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thesis entitled

*A Comparison of Scatchard Analysis
and the Gaussian Distribution Model
to Determine a Conditional Stability
Constant Between the Uranyl Ion and
Humic Substances*

presented by

Richard Alan Geiger

has been accepted towards fulfillment
of the requirements for
Master of Science degree in *Fisheries and Wildlife*

Niles R. Kevern
Major professor

Date July 22, 1985

A COMPARISON OF SCATCHARD ANALYSIS AND THE GAUSSIAN DISTRIBUTION MODEL TO DETERMINE A CONDITIONAL STABILITY CONSTANT BETWEEN THE URANYL ION AND HUMIC SUBSTANCES

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1985

ABSTRACT

A COMPARISON OF SCATCHARD ANALYSIS AND THE GAUSSIAN DISTRIBUTION MODEL TO DETERMINE A CONDITIONAL STABILITY CONSTANT BETWEEN THE URANYL ION AND HUMIC SUBSTANCES

By

Richard Alan Geiger

Naturally occurring aquatic humic substances (HS) are greater controlling factors in the mobilization and distribution of trace metals in blackwater systems of the southeastern USA than most inorganic ions and suspended solids. Ion exchange, photo-oxidation and laser fluorometry were used to determine the maximum binding capacity (BC) of Aldrich humic acid (HA) for UO_2^{2+} and the average conditional stability constant ($\bar{K}'$) between a constant concentration of Aldrich HA and UO_2^{2+} concentrations varying from $5.25 \times 10^{-8}\underline{M}$ to $2.10 \times 10^{-7}\underline{M}$ UO_2^{2+}. The Scatchard analysis over the range of UO_2^{2+}:HS ratios tested is sufficiently linear to predict $\bar{K}'$. A continuous distribution (Gaussian) estimate of $\bar{K}'$ was calculated to compare to the $\bar{K}'$ determined by the single component Scatchard analysis. At UO_2^{2+} concentrations typical of those in aquatic systems of non-uraniferous areas, the estimated $\bar{K}'$ between UO_2^{2+} and Aldrich HA is not substantially different when calculated by Scatchard analysis or Gaussian distribution.

ACKNOWLEDGMENTS

I want to acknowledge the generous assistance of those individuals who made this work possible. Dr. Niles R. Kevern, my major professor, provided invaluable advice and general guidance throughout the development and completion of this study. Drs. John P. Giesy, Jr. and James J. Alberts oversaw the research and furnished encouragement and direction throughout the study. I would like to express my gratitude to two other committee members, Drs. Darrell L. King and David T. Long, for their advice and critical evaluation, particularly during preparation of the research proposal and writing of the thesis. In addition, I want to thank Dr. James M. Tiedje for his advice during the writing of the research proposal and Dr. Michael C. Newman for his critique of the rough draft of the thesis.

I offer my sincerest thanks to the staff and students of the Savannah River Ecology Laboratory. Their friendships over the years contributed to my development as a scientist and an individual. Without their assistance this study may never have been completed.

Several people deserve special recognition. Technical assistance in the study was provided by Ms. Susan Lesnek, Mr. John Bowling and Mr. Fred Stone. Typing of the draft thesis was done by Ms. Jan Moran and Ms. Kathy Tseng. Typing of the final thesis was done by the Savannah River Ecology Laboratory secretarial staff. Ms. Jean Coleman prepared the figures. Technical editing was performed by Ms. Karen Patterson.

Funding was provided through a teaching assistantship from Michigan State University and through contract DE-AC09-76-SR00819 between the University of Georgia and the U. S. Department of Energy.

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INTRODUCTION

Naturally occurring uranium (U) has an atomic weight of 238.03 atomic mass units. The commonly occurring valence states of U in the natural environment are U^{4+} and U^{6+}. Although U^{4+} is found in reduced zones in natural environments (Garrels and Larsen 1959; Bonatti et al. 1971; Kolodny and Kaplan 1973; Brookins 1978; Langmuir 1978), it is not a very soluble chemical species. The U^{6+} ion is generally found in the oxidizing environment of surface waters as the more soluble uranyl ion, UO_2^{2+}, and can actively transport U in the solution phase. Valence electrons in the $5f^3 6d^1 7s^2$ shells can be removed by a strongly electronegative element such as oxygen (O) to form a strongly complexing ion.

With the passage of the Nuclear Waste Policy Act of 1982, state governments have been encouraged to develop regional consortia and to establish site criteria for the disposal of low-level radioactive waste (LLW) at shallow land burial (SLB) sites. Depleted U (i.e. U with less ^{235}U than 0.720%) can be disposed of as LLW in a SLB. Previously, SLB of LLW had been restricted to three companies operating six disposal sites (Francis et al. 1980). Radionuclide migration has been detected at some of these sites; microbial degradation of synthetic or natural organic compounds in the burial trenches may result in formation of ligands that can bind, leach and solubilize to form organo-uranium complexes (Means et al. 1978; Francis et al. 1980; Francis 1981).

The Environmental Protection Agency (EPA) is considering proposing a health guidance concentration of 10 pCi U/ℓ (34 ppb UO_2^{2+} or 1.43×10^{-7} $\underline{M}$ UO_2^{2+}) for drinking water (Cothern et al. 1983). This small concentration may be chronically toxic to aquatic biota continuously exposed to contaminated surface waters.

Of the two hazards associated with U or its compounds, chemical toxicity is considered a greater hazard than radiological because the U compounds obstruct phosphorylation and carbohydrate metabolism, thus causing renal failure (Gindler 1973; Nechay et al. 1980). Ingestion and assimilation of soluble species is one potential toxic pathway of U. With an expected increase in the use of SLB sites around the U.S.A., there is an increasing potential for U to be complexed as a soluble compound and to migrate from these sites. Therefore, it is imperative to understand the factors which control the mobility of soluble U, its ultimate fate and its potential effects.

Uranium is concentrated in several types of igneous and sedimentary rocks (McCormick and Cotter 1964; Osmond 1964; Szalay and Samsoni 1969; Bouwer et al. 1978; Halbach et al. 1980; Johnson et al. 1980; Metzger et al. 1980). During the weathering of granite, U is mobilized from the bedrock, primarily as UO_2^{2+} (Halbach et al. 1980; Tieh et al. 1980). Uranium can also be mobilized during phosphate mining (Osmond 1964; Bouwer et al. 1978; Metzger et al. 1980). During the past twenty years U concentrations in several North American rivers have increased, presumably due to contributions from man's activities (Spaulding and Sackett 1972; Bloch 1980). However, Mangini et al. (1979) suggest that world-wide U concentrations in rivers and lakes are primarily a function of aquatic chemistry rather than input from human activities and are controlled by the inorganic chemistry of the water, not differences in bedrock material or amounts of U leached from phosphate fertilizers.

Several investigators have studied the physical and chemical properties that may increase UO_2^{2+} mobility (Moskvin et al. 1967; Langmuir 1978; Dongarra and Langmuir 1980; Giblin 1980; Giblin et al. 1981). Langmuir (1978) reported the pH range (pH 5.0 - 8.5) of maximum UO_2^{2+} sorption onto most natural colloidal materials, including humic substances, ferric and manganic oxyhydroxides, and clays. This pH range is common to the blackwater streams and swamps of the southeastern U.S.A. (Beck et al. 1974; Reuter and Perdue 1977; Giesy and Briese 1977, 1978a; Alberts and Evans 1979; Alberts and Giesy 1983). These investigators conclude that naturally occurring humic substances are greater controlling factors than most inorganic ions in the mobilization and distribution of trace metals in these soft water systems. Complexation of UO_2^{2+} by naturally occurring aquatic humic substances (HS) may be important in determining the bioavailability of UO_2^{2+} (Jennings and Leventhal 1978) and is influenced by such physico-chemical parameters as pH, Eh, ionic strength, and adsorption to surfaces such as clays or metal oxyhydroxides (Martin et al. 1976; Shelke and Jahagirdar 1979; Li et al. 1980; Kribek and Podlaha 1980; Halbach et al. 1980; Nash et al. 1981).

Comprehensive investigations have been conducted on the chemical structure and function of naturally occurring dissolved organic matter in surface waters. These studies characterize HS, the major component of the dissolved organic matter (Steelink 1977; Perdue 1978; Liao et al. 1982) and describe complexation of trace metals by HS (Jackson

1975; Guy and Chakrabarti 1976; Jackson et al. 1978; Mantoura et al. 1978; Saar and Weber 1982). A decline in productivity can result from the sequestering of nutrients by HS in freshwater systems (Jackson and Hecky 1980). HS are complex organic polymers resulting from the decomposition of plant tissues. They have variable structures which have not been completely defined but are known to be composed of aromatic and aliphatic components with oxygen containing functional groups, particularly carboxyl (-COOH) and phenolic hydroxyl (ϕ-OH) (Rashid and King 1970; Schnitzer and Khan 1972; Beck et al. 1974; Borggaard 1974; Reuter and Perdue 1977; Choppin and Kullberg 1978; Li et al. 1980; Christman and Gjessing 1983). These functional groups can protonate and dissociate in the pH range (pH 3.0 - 9.0) common in natural surface waters (Gamble 1970; Choppen and Kullberg 1978; Saar and Weber 1982). Aquatic HS are important in southeastern U.S.A. surface waters because their abundance and binding capacities make them potential controlling factors in the biogeochemical cycles of inorganic ions in these waters (Beck et al. 1974; Schindler and Alberts 1974; Casagrande and Erchull 1976; Giesy and Briese 1977, 1978a; Reuter and Perdue 1977; Alberts and Evans 1979).

The strong association between U and organic matter has been known for some time (Moore 1954; Szalay 1954, 1958, 1964a,b, 1969; Breger et al. 1955a,b; Szalay and Samsoni 1969). Langmuir (1978) mentions the importance of the UO_2^{2+}-organic matter complexes in the geochemical cycling process and cites previous work on the subject (Germanov and Panteleyev 1968; Haji-Vassiliou and Kerr 1973; Pauli 1975).

In previous investigations a major problem has been the lack of a rapid, accurate analytical technique to detect the concentration of U bound to aquatic HS. Many techniques are not sufficiently sensitive at small concentrations of U. Using thermodynamic simulation models to predict the speciation of UO_2^{2+} in surface waters is desirable, but to accomplish this the thermodynamic interactions of UO_2^{2+} with organic and inorganic ligands must be known. While the literature is replete with information on the inorganic solubility and complexation chemistry of UO_2^{2+} (Langmuir 1978; Dongarra and Langmuir 1980), much less is known about the thermodynamic interactions between UO_2^{2+} and aquatic HS.

The hypothesis tested in this study was that environmental concentrations of uranium (U) will bind tightly to the aquatic humic component under conditions representative of natural surface waters of the southeastern U.S.A. To test the hypothesis it was necessary (1) to develop a rapid, sensitive method for quantifying U concentrations of inland surface waters, (2) to determine maximum binding capacity (BC) of aquatic HS for the uranyl ion (UO_2^{2+}), (3) to determine the average conditional stability constant ($\bar{K}'$) between UO_2^{2+} and aquatic HS under simulated natural conditions in the laboratory using both the Scatchard analysis and a Gaussian model, and (4) to evaluate which of the two analytical methods more accurately represents the $\bar{K}'$ at concentrations of UO_2^{2+} and HS typical of surface waters of the southeastern U.S.A.

MATERIALS AND METHODS

Aldrich humic acid (Aldrich Chemical Co., Milwaukee, WI: H-1675-2, Lot 082091) used in this study was purified by the methods of Landrum and Giesy (1981). Briefly, the HA was dissolved in 0.1 N NaOH, centrifuged and the supernatant decanted. Supernatant pH was reduced to 2 with 6 N HCl, then left undisturbed for 18 h. The resulting HA precipitate was centrifuged and the supernatant discarded. The solid was dissolved in 0.1 N NaOH, transferred to a beaker and stirred at room temperature for 18 h. This solution was centrifuged and the precipitation, resolution and centrifugation were repeated twice. The final precipitate was dissolved into 0.1 N NaOH and the solution pH adjusted to 7 with 6 N HCl. The solution was transferred to dialysis bags (Fisher Scientific, Pittsburgh, PA) and dialysed against deionized water until the conductivity of the water outside the bags was 10 µmhos. Following dialysis, the solution was refrigerated in polyethylene bottles.

The dry weight and ash free dry weight of the purified HA stock solution were determined on 5 replicate samples. Eighty (80) ml aliquants of the extracted solution were dried at 85°C. Average dry weight of the 5 replicates was 3.53 ± 0.0002 mg/ml. Average ash free dry weight (air atmosphere) was 1.18 ± 0.003 mg/ml. Ash weight accounted for 66.6% of the average dry weight.

In solutions containing low concentrations of metals, loss of elements to the surface of glassware can result in large experimental errors (Giesy and Paine 1977). To minimize adsorptive losses to the glass surfaces, all glassware was thoroughly washed, rinsed and treated with 1% Prosil-28® (PCR Research Chemicals Inc., Gainesville, FL). Glassware was retreated after every tenth use.

Uncomplexed UO_2^{2+} was separated from that complexed to Aldrich HA by Chelex-100® chelating resin (Na^+ form; BioRad Laboratories, Richmond, CA). Chelex-100® is a weakly acidic ketoiminocarboxylic cation exchange resin with properties similar to those of naturally occurring HA. Separations were conducted in 10 ml glass columns with fritted glass bottoms (BioRad Laboratories, Richmond, CA) over which a 0.22 μm acetate filter (Millipore Corp., Bedford, MA) was fitted.

A 0.01 M acetate (Ac^-) buffer solution was prepared by combining 0.01 M (0.82 g/ℓ) sodium acetate (NaOAc) and 0.017 M (1.00 ml) acetic acid (HOAc). The pH was adjusted to 4.5 with 1.0 M or 0.1 M Na_2CO_3. Under the conditions of the study (pH 4.5 and 0.01 M Ac^-) and at 4.2×10^{-6} M UO_2^{2+}, 95.1% of the UO_2^{2+} should be complexed by the Ac^- in the absence of HA (Table 1). GEOCHEM, a thermodynamic equilibrium model for natural water systems (Sposito and Mattigod 1979), was used to predict equilibrium concentrations of inorganic and simple UO_2^{2+}-organic species. Relative to humics, Ac^- weakly complexes UO_2^{2+}; GEOCHEM accounts for the competition between Ac^- and HA for UO_2^{2+} ions.

For each separation, Chelex-100® resin swelled in the Ac^- buffer (pH 4.5) and 2 ml (0.8 meq) was placed in the column. Overlying Ac^- buffer was removed and the column filled with the appropriate test solution. The column was then attached to a 500 ml Mariotte flask

Table 1. Primary distribution of metals and ligands in test solution (pH 4.5; 0.01 M Ac^-)

Metal/Ligand	Distribution	Percentage*
Na^+	Free metal	99.5
	Bound with Ac^-	0.5
UO_2^{2+}	Free metal	4.5
	Bound with Ac^-	95.1
	Bound with OH^-	0.5
Ac^-	Free ligand	39.2
	Bound with Na^+	0.2
	Bound with H^+	60.6
NO_3^-	Free ligand	99.8
	Bound with Na^+	0.2

* Percentage may not add to 100.0 due to rounding error

arrangement containing additional test solution which ensured a consistent flow rate.

Before separations were conducted, flow rate through the exchange column was optimized so that unbound UO_2^{2+} was quantitatively removed from the solution without significantly altering the equilibrium between uncomplexed and complexed UO_2^{2+} (Giesy 1980). To do this, 500 ml of 4.2×10^{-6} $\underline{M}$ UO_2^{2+} solution was prepared in 0.01 $\underline{M}$ Ac^-buffer (pH 4.5) and 3.5 mg C/ℓ dialyzed Aldrich HA added. An identical solution without the Aldrich HA was prepared as a reference solution. The ion exchange column was filled with the UO_2^{2+}-HA solution. Approximately 10 ml (5 bed volumes or BV) of solution were eluted and discarded. A minimum flow rate was set with an adjustable screw clamp, and at least one BV of solute was eluted and discarded. Then a 15 ml aliquot was collected, the flow rate was increased, measured and recorded, and at least one more BV of solute was eluted and discarded before the second sample aliquot was collected. This procedure was repeated until either the flow rate was maximized or no increase in the amount of UO_2^{2+} eluting from the column was detected. The procedure was repeated for the UO_2^{2+} solution without the HA.

UO_2^{2+} standards were prepared in pH 4.5 Ac^- buffer from reference standards (UO_2^{2+} as $UO_2(NO_3)_2 \cdot 6H_2O$; Anderson Laboratories, Fort Worth, TX). The UO_2^{2+}:HA ratio was varied by maintaining a constant HA concentration of 3.5 mg C/ℓ for each test solution and adding UO_2^{2+} to attain concentrations from 4.2×10^{-8} $\underline{M}$ to 2.1×10^{-5} $\underline{M}$ UO_2^{2+} (Table 2). The optimal flow rate was set and 60 ml (30 BV) of the test solution was eluted and discarded before a 15 ml aliquot was taken for each UO_2^{2+}:HA ratio.

Table 2. Experimental flow rates of various uranyl concentrations (M) through Chelex-100® ion exchange resin column (0.01 M Ac^- and 3.5 mg C/ℓ at pH 4.5)

UO_2^{2+} (M)	Flow Rate (mls/min)
$4.20 \times (10)^{-8}$	18.0
$5.25 \times (10)^{-8}$	18.0
$6.30 \times (10)^{-8}$	18.0
$7.35 \times (10)^{-8}$	18.8
$8.40 \times (10)^{-8}$	18.6
$1.05 \times (10)^{-7}$	18.5
$1.26 \times (10)^{-7}$	18.0
$1.68 \times (10)^{-7}$	18.5
$2.10 \times (10)^{-7}$	17.8
$3.15 \times (10)^{-7}$	18.2
$4.20 \times (10)^{-7}$	18.0
$6.30 \times (10)^{-7}$	18.0
$8.40 \times (10)^{-7}$	17.8
$1.05 \times (10)^{-6}$	17.8
$1.26 \times (10)^{-6}$	17.8
$1.68 \times (10)^{-6}$	17.7
$2.10 \times (10)^{-6}$	17.7
$3.15 \times (10)^{-6}$	17.7
$4.20 \times (10)^{-6}$	17.9
$1.05 \times (10)^{-5}$	17.5
$2.10 \times (10)^{-5}$	18.0

Organic matter (Aldrich HA) interferes with laser fluorometry and was therefore eliminated by photo-oxidation. Chemical oxidation by potassium permanganate was not used because of interference from Mn^{2+} and changes in sample volume. All samples to be analyzed for U, including those without HA, were photo-oxidized 2 h in a xenon arc ultraviolet photo-oxidation unit (La Jolla Scientific Co., La Jolla, CA). Besides eliminating interference from HA, this oxidation procedure also destroyed the C-C bonds of the Ac^- buffer which also may have interfered with the analysis. Photo-oxidized samples were neutralized to approximately pH 7 with a small volume of Na_2CO_3 and diluted (5 to 10 times) so that the sample concentration fell within the selected detection range of the instrument.

Uranium concentrations were determined with a Scintrex Model UA-3 Uranium Analyzer (Scintrex Ltd., Toronto, Canada) which uses pulsed laser-induced fluorescence to detect ng (ppb) quantities of U. The analyzer was zeroed and a cuvette containing 5 ml of sample was placed in the sample compartment. The photomultiplier tube (PMT) was activated, any residual signal was nullified and the PMT turned off. Five hundred µl (0.5 ml) of Fluran®, a proprietary pyrophosphate buffer (Scintrex Ltd., Toronto, Canada), was then added to the 5 ml sample which was thoroughly mixed and returned to the sample compartment. Fluran® strongly complexes various UO_2^{2+} species which may be present in a sample into a single species with a high luminescent yield. The PMT was reactivated and a sample reading recorded when the meter deflection stabilized (10 - 20 sec). If the meter deflection was either off-scale or minimal, a more appropriate range was selected and the entire procedure from the initial zeroing was repeated.

After a value for the sample was obtained, 25 or 50 µl of 1.05 x 10^{-6} $\underline{M}$ UO_2^{2+} solution were added to the aliquot and mixed; the volume added depended on the initial meter deflection. The PMT was reactivated and a reading taken of the meter deflection; sample UO_2^{2+} concentration was calculated (Equation 1). This standard addition technique gives more accurate data than the semi-quantitative calibration curve technique (Scintrex N.D.), but the principal disadvantage of the standard addition method is that it is more time consuming since it must be done for each sample.

$$Z = \frac{D_1}{D_1 - D_2} \cdot \frac{a}{b} \cdot A \qquad (1)$$

where: Z = U concentration of sample aliquot

D_1 = meter deflection due to sample

D_2 = meter deflection due to sampling plus standard addition

a = volume of standard addition (25 or 50 µl)

b = volume of sample aliquot (5 x 10^3 µl)

A = U concentration of standard solution (1.05 x 10^{-6} $\underline{M}$ UO_2^{2+}).

The total, unbound and bound U concentrations were then used to compare conditional stability constants by Scatchard and Gaussian methods (see Theoretical Considerations).

Linear and nonlinear regressions for estimating BC and the Scatchard parameters were computed using the general linear model (GLM) and nonlinear least square techniques (NLIN) of SAS (Helwig and Council 1979) and Marquardt nonlinear least squares procedures. The Gaussian estimates of the conditional stability constants were obtained with KINFIT4 (Dye and Nicely 1971) and a driver program written by J. P. Giesy (Giesy, pers. comm.)

THEORETICAL CONSIDERATIONS

Stability Constants

The affinity of a metal for a particular ligand is represented by a stability (formation) constant, K. By definition, K is a competitive reaction between a metal ion and protons for binding sites on the ligand, therefore, it is pH dependent (Equations 2 and 3).

$$M^{n+} + LH_x <=> MLH_{x-n} + nH^+ \tag{2}$$

$$K = \frac{(MLH_{x-n})\ (H^+)^n}{(M^{n+})\ (LH_x)} \tag{3}$$

where: K = stability constant

M^{n+} = metal ion with n deficient electrons; M_f will be used in subsequent equations to denote the unbound metal concentration

H^+ = hydrogen ion

L = organic ligand

x = number of protons released from the complexing ligand in order to complex the metal ion; L_f will be used in subsequent equations to denote ligand L with proton deficiency x.

There are numerous HS functional groups and several acid dissociation constants can be measured (Giesy and Alberts 1984), which indicates that a number of complexation reactions can take place simultaneously. Even if the HS functional groups involved in binding an ion such as UO_2^{2+} were very homogeneous, a range of complexes with different stability constants would be expected due to polyelectric effects of sequential site filling and the formation of both mononuclear and polynuclear complexes. For these reasons conditional stability constants (rather than stability constants) are used to describe the strength of complexes at constant pH (Equation 4).

$$K'_i = \frac{[ML_i]}{[M_f]\ [L_f]} \qquad (4)$$

where: K'_i = conditional stability constant for complex type i corrected for proton deficiency

$[ML_i]$ = molar concentration of metal complexed to ligand type i

$[M_f]$ = molar concentration of uncomplexed metal; this includes both ionic species (M^{n+}) and hydrated ionic species ($M^{n+} \cdot yH_2O$)

$[L_f]$ = molar concentrations of uncomplexed ligand of type i; all ligand types are not necessarily complexed to <u>any</u> metal, whether of interest or not.

Discrete Multiligand Models

In a heterogenous mixture of binding sites, an average stability constant ($\bar{K}$) can be defined (Equation 5; notation of Perdue and Lytle 1983).

$$\bar{K} = \frac{\Sigma_i\ [ML_i]}{[M_f]\Sigma_i[L_i]} = \frac{\Sigma_i\ K_i\ [L_i]}{\Sigma_i\ [L_i]} \qquad (5)$$

where: $[L_i]$ = molar concentration of uncomplexed ligand of type i.

The average conditional stability constant ($\bar{K}'$) is described by Equations 6 - 8.

$$\bar{K}' = \frac{\Sigma_i \; [ML]}{[M_f] \; \Sigma_i \; [H_{xi} \; L_i]} \tag{6}$$

where: $[H_{xi} \; L_i]$ = concentration of protonated ligand of type i (x is the number of protons released from complexed ligand L_i).

$$\bar{K}' = \frac{\Sigma_i \; K'_i \; [H_{xi} L_i]}{\Sigma_i \; [H_{xi} \; L_i]} \tag{7}$$

$$\bar{K}' = \frac{[M_T] - [M_f]}{[M_f] \; ([L_T] - [M_T] + [M_f])} \tag{8}$$

where: $[M_T]$ = total molar concentration of metal
$[L_T]$ = total molar concentration of ligand; all types of sites.

Stability constants have been calculated for metals complexed by ligands of known molecular weight and structure (Buffle et al. 1977). For the organic ligands of unknown structure which occur in natural surface waters (Stevenson and Ardakani 1972; Gardiner 1974), stability constants are much more difficult to calculate because of the inability to calculate molar ligand concentrations (Stevenson 1977) or to define the complexes as mononuclear or polynuclear and to describe properties of each type of complex. Instead, average conditional stability constants have been calculated by comparing the metal binding capacity of a ligand of unknown structure to the binding capacity of the same metal with a reference ligand of known structure (Equation 9).

$$\bar{K}' = \frac{\Sigma_i\, K'_i \left[\dfrac{H_{xi}L_i}{H_{xr}L_r}\right]}{\Sigma_i \left[\dfrac{H_{xi}L_i}{H_{xr}L_r}\right]} \qquad (9)$$

where: $H_{xr}L_r$ = concentration of protonated reference ligand.

The Schubert ion exchange method (Schubert 1948; Miller and Ohlrogge 1958; Randhawa and Broadbent 1965; Schnitzer and Hansen 1970) estimates an average conditional stability constant ($\bar{K}'$) for a metal-ligand system by measuring the distribution of a metal between a solute and solid phase both in the absence and the presence of a complexing agent. This method does not allow the ligand concentration to vary; only 1:1 metal-ligand complexes can be measured. For ligand mixtures such as HS found in the blackwater streams of the southeastern U.S.A., an average stability constant ($\bar{K}$) is estimated (Clark and Turner 1969; Gamble et al. 1970; Schnitzer and Hansen 1970; Stevenson and Ardakani 1972; Wahlgren et al. 1972; Beck et al. 1974; Crosser and Allen 1977; Giesy et al. 1977; Giesy et al. 1978; Giesy and Briese 1980; Giesy 1980). The Bjerrum method (van den Berg and Kramer 1979) was developed specifically for determination of $\bar{K}'$ for compounds such as HS (Stevenson 1977), if one type of binding site is present, but also provides an average stability constant, $\bar{K}$.

The Scatchard analysis, another method of estimating $\bar{K}'$ is a discrete model which results in a straight line if only one type of metal binding site is present. (Scatchard 1949; Mantoura and Riley 1975; Guy and Chakrabarti 1976; Mantoura et al. 1978; Giesy 1980; Sposito 1981; Saar and Weber 1982). Scatchard analyses do not indicate if specific types of complexes form nor if 1:1 complexes with different stability constants exist. However, average stability constants can be estimated from Equations 10 - 16.

$$v_i = \frac{[ML_i]}{[L_T]} \qquad (10)$$

where: v_i = ratio of concentration of complexed ligand of type i to concentration of total ligand; all types of sites

$[L_T] = [ML_i] + [H_{xi}L_i]$.

$$v_i = \frac{K'_i\ [M_f]}{1 + K'_i\ [M_f]} \qquad (11)$$

When summed over all types of sites present in the mixture, $\bar{v}$ is the ratio of metal bound to the total number of sites present (Equation 12).

$$\bar{v} = \frac{\Sigma_i\ v_i [L_{Ti}]}{\Sigma_i\ [L_{Ti}]} \qquad (12)$$

where: $[L_{Ti}]$ = total molar concentration of ligand type i.

$$\bar{v} = \Sigma_i \left(\frac{K'_i\ [M_f]}{1 + K'_i\ [M_f]} \cdot \frac{[L_{Ti}]}{[L_T]} \right) \qquad (13)$$

$$\bar{K}' = \frac{1}{[M_f]} \left(\frac{\bar{v}}{1 - \bar{v}} \right) \qquad (14)$$

If one type of site is assumed, the Scatchard relationship can be expressed by Equation 15.

$$\bar{v} = \left(\frac{K'_i\ [M_f]}{1 + K'_i\ [M_f]} \cdot \frac{[L_1]}{[L_T]} \right) \qquad (15)$$

where: L_1 = total molar concentration of one ligand type.

$$\frac{\bar{v}}{[M_f]} = K'_i\ (n_i - \bar{v}) \qquad (16)$$

where: n_i = number of binding sites per molecule of HS.

Graphical interpretation of Equation 16 is given by a plot of $\frac{\bar{v}}{[M_f]}$ as a function of $\bar{v}$ and can provide an estimate of n_i and K'_i. It is assumed that only one type of binding site is present if the plot is linear; if the plot is curvilinear, the metal is bound by more than one type of site. When more than one type of binding site is indicated, values for n_i and K'_i cannot be resolved graphically by extrapolating linear regions of the curve because each type of site contributes to the nearly linear portions of both ends of the curve (Giesy 1980). Values for n_i and K'_i can be estimated by computation using iterative nonlinear least squares techniques. Generally two types of metal binding sites (n_1 and n_2) and consequently two conditional stability constants (K'_1 and K'_2) result from a curvilinear Scatchard plot. One type of site (n_1) is less common than the other type (n_2), but forms a stronger bond with the metal ion. The conditional stability constant (K') will be greater for the type of site that has fewer available binding sites.

The Scatchard analyses for the interaction of metals with HS are generally curvilinear, as seen in this study. In an attempt to more accurately describe this relationship, a number of authors have used Scatchard relationships which include multiple types of sites. If two discrete types of sites are assumed, the relationship is described by Equation 17.

$$\bar{v} = \left(\frac{K'_1 [M_f]}{1 + K'_1 [M_f]} \cdot \frac{[L_{T1}]}{[L_T]} \right) + \left(\frac{K'_2 [M_f]}{1 + K'_2 [M_f]} \cdot \frac{[L_{T2}]}{[L_T]} \right) \qquad (17)$$

While this relationship successfully describes the empirical data, it does not necessarily mean that there are actually two discrete types of sites present. Perdue and Lytle (1983) show that the average conditional stability constants derived in this way are not constant but vary as a function of the total metal to total ligand ratio (M_T:L_T). Hence the estimates of the average conditional stability constants derived from two component Scatchard analysis are useful only in the range of M_T:L_T for which they were determined and cannot be extrapolated to other M_T:L_T ratios. Because of the minimum detection limits in the analytical techniques used by many researchers today, the experimental metal:ligand ratio generally must be much greater than in natural surface waters.

Continuous Multiligand Models

Average stability constants ($\bar{K}$) calculated for a multiligand mixture such as aquatic HS are not constant because the reference ligand will most certainly have a different affinity for the metal ion than will the experimental ligand; consequently the ratio of the two ligands will vary as the overall metal to ligand ratio changes in the solution. It follows that the average conditional stability constant ($\bar{K}'$) would vary with changes in the composition of the solution, and therefore should not be considered a constant at all (Perdue and Lytle 1983). However, with excess amounts of reference and experimental aquatic HS or extremely low concentrations of metal, the overall metal to ligand ratio should not change perceptibly. On the other hand, the analytical technique may require larger metal concentrations to be used which are not characteristic of those found in the natural environment (Perdue and Lytle 1983).

The Scatchard analysis used to evaluate the metal complexing ability of HS in this and other studies (Mantoura and Riley 1975; Guy and Chakrabarti 1976; Mantoura et al. 1978; Giesy 1980; Kribek and Podlaha 1980; Li et al. 1980; Alberts and Giesy 1983; Shuman et al. 1983) has been criticized for its lack of rigor in multiligand systems. Perdue and Lytle (1983) object to the use of the Scatchard equation for multiligand binding, principally because (1) it does not meet the requirement for a known molar (M) concentration of the ligand and (2) the four curve-fitting parameters can lead to the erroneous conclusion that aquatic HS contain only two nonidentical binding sites. Perdue and Lytle (1983) demonstrate that the average conditional stability constant derived from a four parameter Scatchard model, which assumes two classes of sites, is not independent of the M_T:L_T ratio. They also demonstrate that parameters which are determined by using a curve-fitting technique are average stability constants for two classes of sites, each of which can be made up of a number of similar but nonidentical metal binding sites. To be useful in predicting organic-inorganic relationships in surface waters, Perdue and Lytle (1983) indicated that stability constants must be derived under metal:ligand ratios similar to those encountered in surface waters.

MINEQL is a computer program for the calculation of chemical equilibria in aqueous systems. In their study, Perdue and Lytle (1983) incorporated data from the MINEQL simulation program for a hypothetical continuum, in this case the Gaussian distribution. They noted that a continuous distribution model may be used to predict the extent of metal complexation at metal concentrations representative of those in the environment from laboratory results obtained at much higher metal concentrations (Perdue and Lytle 1983).

Scheinberg (1982) noted that the measurements must be made at equilibrium and that the molarities of both metal and ligand must be known. If these conditions are not met, a Scatchard analysis of the results of metal-ligand binding may be of heuristic value, but will not yield the number and stability constants of the binding sites on the binding molecule.

Hunston (1975) compared Scatchard analysis to a continuous distribution of binding (i.e., stability) constants, which does not require the assumption of a functional form for the distribution or knowledge of the number of classes of independent binding sites. The general continuous distribution model is not limited by the number of parameters as is a more discrete distribution of binding constants. When values of n_i are plotted as a function of the binding site constant, the resulting distribution of the binding site constants can be used to characterize the binding reaction. The distribution of binding constants is best characterized by an average binding constant and a standard deviation, which serves as a measure of the dissimilarity of the binding constants (Hunston 1975).

Posner (1966) reported that proton binding by HS was efficiently described by a continuous multiligand distribution model. While no data have been published that suggest HS have a Gaussian distribution of metal-binding ligands, physical and chemical binding characteristics of monovalent and divalent cations should be sufficiently alike to pursue UO_2^{2+}-aquatic HS binding described by a continuous distribution model.

The Gaussian distribution is a symmetrical two parameter frequency distribution described by a central tendency or mean and a measure of dispersion around that mean, which is referred to as the standard deviation. The parametric mean (μ) and standard deviation (σ) are related when combined with the discrete Scatchard model (Equation 18).

$$\frac{[ML]}{[L_T]} = \bar{v}_{calc} = \frac{1}{\sigma\ 2\pi} \int_{-\infty}^{\infty} \left(\frac{[M_f]10^{\log K'}}{1+[M_f]10^{\log K'}} \right) e^{-0.5 \left(\frac{\mu - \log K'}{\sigma}^{2} \right)} \cdot d \log K' \tag{18}$$

where: $\bar{v}_{calc}$ = empirical ratio $ML:L_T$ at each point in a titration

$\infty = \mu - 4\sigma$

$-\infty = \mu + 4\sigma$.

This frequency distribution can be evaluated numerically by substituting μ and σ such that the residual sums of squares (RSS) is minimized (Equation 19).

$$RSS = \frac{\Sigma_i (\bar{v}_{calc} - \bar{v}_{exp})^2}{\bar{v}_{exp}} \tag{19}$$

where: $\bar{v}_{calc}$ = calculated $\bar{v}$

$\bar{v}_{exp}$ = expected theoretical $\bar{v}$.

A Gaussian distribution model has certain advantages over a discrete two ligand model. First, the symmetrical Gaussian function makes it theoretically possible to define the shape of the curve at higher metal concentrations ($\bar{v} \geq 0.5$) and to extrapolate to lower concentrations ($\bar{v} \rightarrow 0$). Second, from a purely empirical point of view, the Gaussian distribution model with two curve-fitting parameters is less restrained than the two component Scatchard equation with four curve-fitting parameters (Perdue and Lytle 1983). Iterative calculations optimize several parameters (log K' or $\bar{K}'$, $\sigma_{\log \bar{K}'}$, and $\bar{v}$) and are necessary in both the Gaussian distribution and Scatchard models. Like the Scatchard technique, the continuous distribution model is not able to reduce multiligand chemical equilibrium systems to thermodynamic equilibrium constants (Perdue and Lytle 1983).

Binding Capacity

To determine the conditional stability constants of individual metal-ligand complexes or an average conditional stability constant for a ligand mixture, one needs to know the amount of uncomplexed (M_f) and complexed (ML) metal as well as the total ligand concentration (L_T) and the amount of uncomplexed (L_f) and complexed (ML) ligand. This is relatively easy for single ligand systems, when the structure and molecular weight of the ligand are known. In the case of HS no exact molecular weight can be assigned. Some authors have assigned nominal molecular sizes based on ultrafiltration (Gjessing 1970; Kwak et al. 1977; Giesy and Briese 1977, 1978a,b; Giesy et al. 1977; Giesy and Paine 1977; Giesy and Alberts 1984).

The molar concentration of the total number of metal binding site available has to be known.

The maximum binding capacity (BC) is the metal complexing capability by active anionic sites in a solution (Miller and Ohlrogge 1958; Zunino et al. 1972). BC is not necessarily equal to the total number of sites because stereochemical configurations of the polymeric humic ligand may reduce the availability of some sites for metal binding. BC is defined for specific environmental conditions (e.g., pH, ionic strength, temperature) and in surface waters can be either generic (i.e., total cationic complexation by both inorganic and organic anionic components of surface waters) or component specific (i.e., BC measured for selected inorganic or organic anionic components).

There are several ways to determine BC. The most direct technique is to titrate a solution of ligand with a metal and determine the relative concentrations of complexed versus uncomplexed metal such as mg UO_2^{2+}/ℓ of HS. The number of metal binding sites can be expressed as $\underline{M}$/mg or $\underline{M}/\ell$. This technique is imperfect because the humic material can be precipitated at higher concentrations of metal, which may cause stereochemical changes that alter the number of available sites measured under these conditions (Reuter and Perdue 1977).

An alternative method is to calculate the molar concentration of binding sites (n_i) from a Scatchard analysis. This can be done by plotting $\bar{v}$/mg HS (Alberts et al., in press). Because of the limitations of the Scatchard technique this method was not used in this study.

A third technique, which eliminates a number of problems associated with both the previous techniques, is to titrate the natural water or solution of interest with UO_2^{2+} solution (Alberts et al., in press). The maximum BC is determined by fitting a first order saturation model to the data (Figure 1).

A fourth method of determining BC is to calculate the maximum reduction of UO_2^{2+} concentrations by titrating UO_2^{2+} standard solutions with natural water or solution of interest (Giesy et al. 1978; Equation 20).

$$BC = \frac{(C_i)(V_s)-(C_f)(V_s + V_T)}{V_T} \tag{20}$$

where: BC = binding capacity (µg-atoms UO_2^{2+}/ml solution)

C_i = initial free UO_2^{2+} ion concentration (µg-atoms UO_2^{2+}/ml solution)

V_s = volume of UO_2^{2+} standard solution (ml)

C_f = final free UO_2^{2+} ion concentration (µg-atoms UO_2^{2+}/ml solution)

V_T = volume of titrant added (ml of natural water or solution of interest).

Figure 1. Observed versus predicted concentrations of uranium complexed by Aldrich humic acid as a function of total uranium added to the solution (M; pH 4.5; 0.01 M Ac^-).

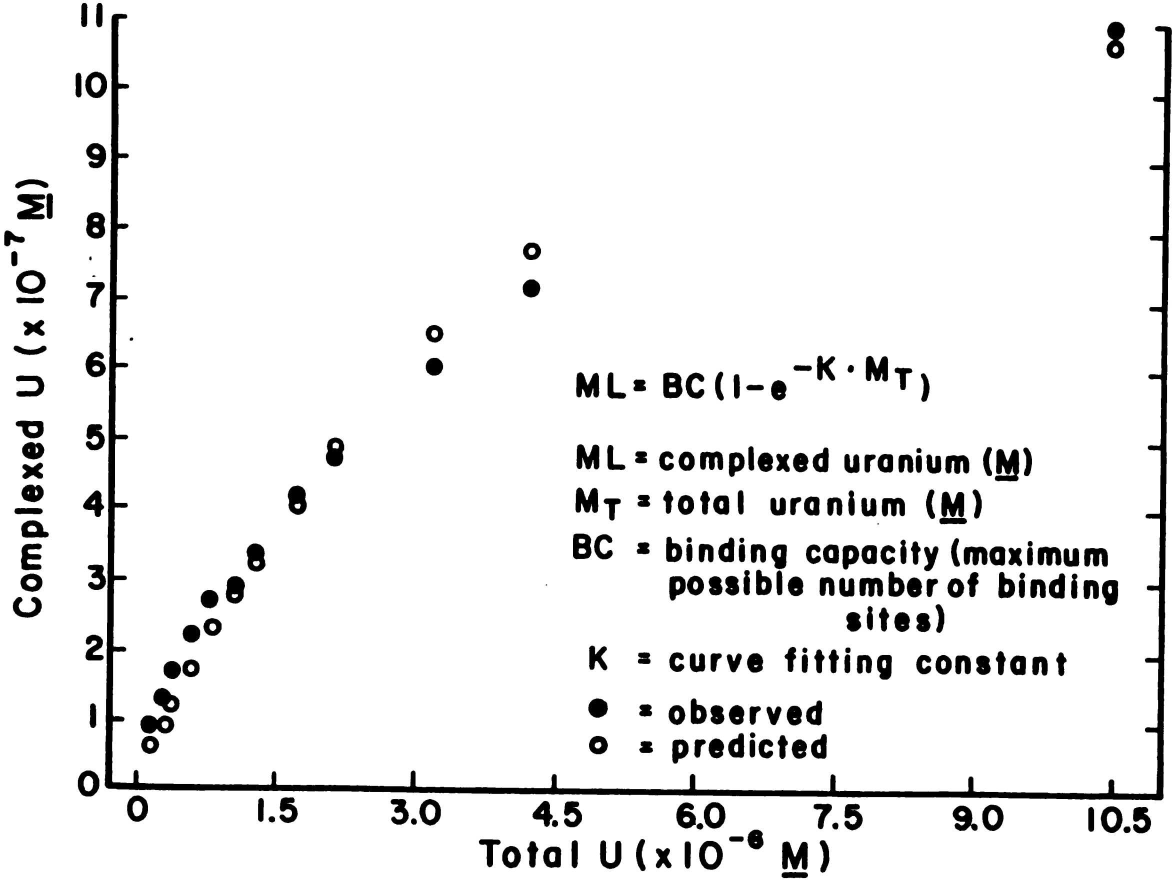

Complexed U (x 10^{-7} M)
Total U (x 10^{-6} M)
0
1
2
3
4
5
6
7
8
9
10
11
0
1.5
3.0
4.5
6.0
7.5
9.0
10.5
ML = BC(1 − e$^{-K \cdot M_T}$)
ML = complexed uranium (M)
M$_T$ = total uranium (M)
BC = binding capacity (maximum possible number of binding sites)
K = curve fitting constant
● = observed
○ = predicted

Figure 1.

In this study complexed and humic-bound UO_2^{2+} were separated by column ion exchange techniques. Therefore, kinetics of UO_2^{2+}-HS reactions could affect calculated BC. Because the BC of UO_2^{2+}-HS solutions are reaction dependent, optimal flow rates must be established for experiments which use ion exchange columns. This flow rate should sustain equilibrium conditions in the column. In this study, the flow rate was optimized to prevent decoupling of humic-bound UO_2^{2+} ions by Chelex-100® resin which has a greater affinity than humics for UO_2^{2+} ions. A flow rate of greater than 16 ml of UO_2^{2+}-Aldrich HA solution/min is required to minimize potential disequilibrium conditions as the UO_2^{2+}-HS pass through the resin column (Figure 2). This elution rate is considerably faster than that reported (4-6 ml/min) for similar studies with divalent transition metals (Giesy 1980). The difference may be attributed to (1) relative affinities of different types of ion exchange resins for the same model or (2) relative affinity of the same resin for different metals. Chelex-100® is reported to have an affinity for the UO_2^{2+} ion which is exceeded only by the affinity for Hg^{2+} and Cu^{2+} ions (BioRad Laboratories 1972). This strong affinity for the UO_2^{2+} ion compared to other divalent cations necessitates a rapid flow rate through the resin column in order to maintain equilibrium conditions. If the flow rate through the column is too slow, Chelex-100® resin can remove UO_2^{2+} ions from UO_2^{2+}-HS complexes (Figure 2). However, a flow rate of 16 ml/min allows quantitative removal of the free UO_2^{2+} (Hathaway and James 1975).

BC is estimated from a plot of the bound UO_2^{2+} as a function of the total UO_2^{2+} concentration. A least squares approximation of BC can be obtained (Equation 21).

$$[ML] = BC(1-e^{-\lambda[M_T]}) \tag{21}$$

where: $[ML]$ = humic bound UO_2^{2+} ($\underline{M}$)

BC = maximum possible number of binding sites

$[M_T]$ = total UO_2^{2+} added to solution ($\underline{M}$)

λ = curve fitting constant.

Figure 2. Concentration of uranium (M) eluted through a Chelex-100® ion exchange resin column as a function of flow rate of the solution through the column (ml/min; pH 4.5; 0.01 M Ac^-; 3.5 mg C/ℓ, dialyzed Aldrich HA).

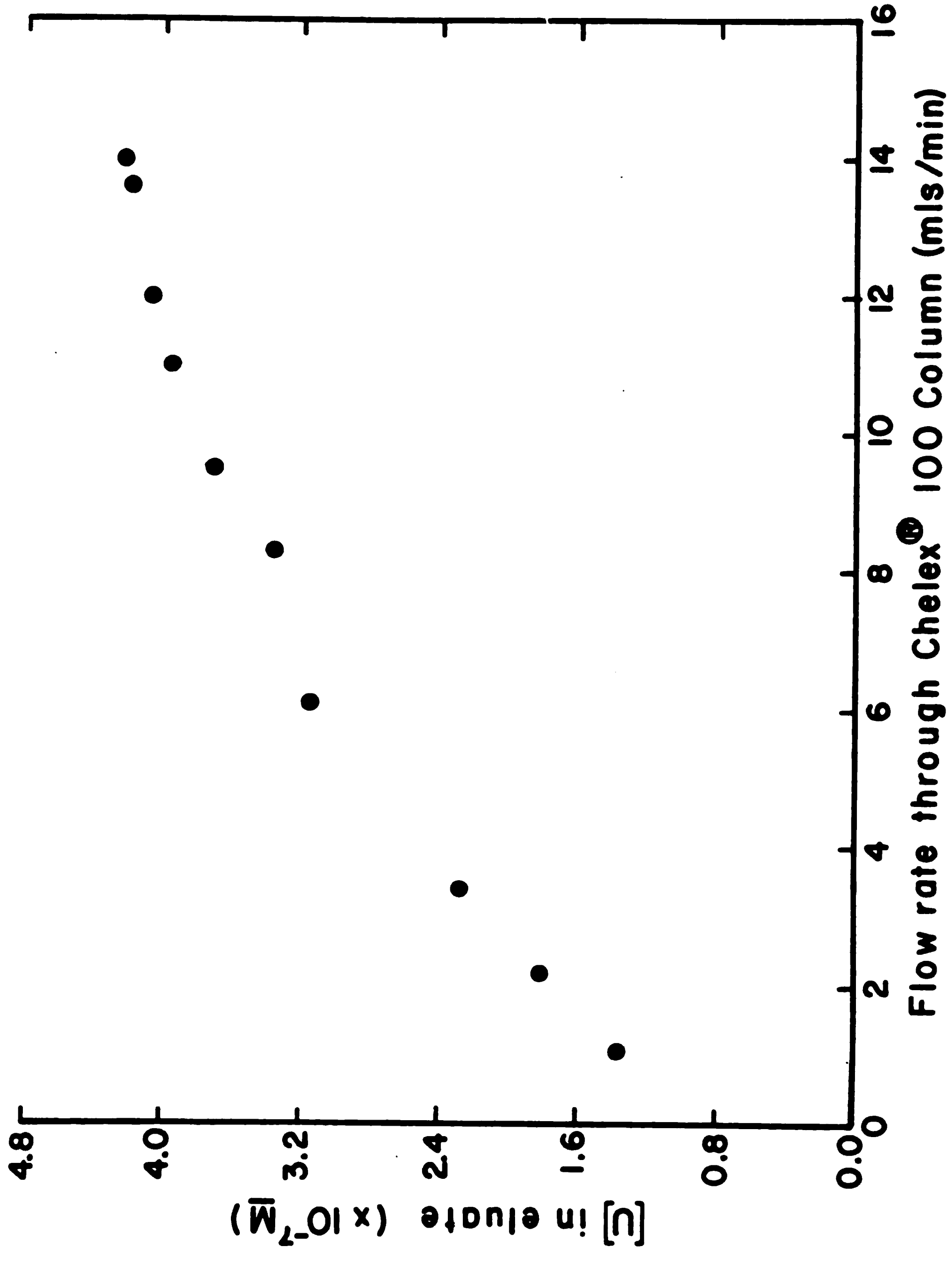

[U] in eluate (x 10^{-7} M)
0.0
0.8
1.6
2.4
3.2
4.0
4.8
0
2
4
6
8
10
12
14
16
Flow rate through Chelex® 100 Column (mls/min)

Figure 2.

RESULTS

The maximum binding capacity (BC) of Aldrich HA was determined with uranium concentrations from 3.15×10^{-7} $\underline{M}$ to 2.10×10^{-5} $\underline{M}$ UO_2^{2+} (Figure 1). The BC of a 3.5 mg C/ℓ solution was estimated to be 1.14×10^{-6} $\underline{M}$ UO_2^{2+} with an asymptotic standard error of 5.0×10^{-8} $\underline{M}$ UO_2^{2+} (Table 3). The 95% confidence interval (CI) is $\pm 1.1 \times 10^{-7}$ $\underline{M}$ UO_2^{2+}. A highly significant F-statistic was calculated for the regression of the concentration of UO_2^{2+} eluted through the ion exchange column as a function of the concentration of UO_2^{2+} added to the column ($F_{2,14(0.05)} = 1118$; $P \leq 0.0001$). The BC of Aldrich HA was thus 4.8×10^{-7} $\underline{M}$ UO_2^{2+}/mg C.

The Scatchard analysis was non-linear over the entire $[M_T]:[L_T]$ range titrated (Figure 3). The estimate of the average conditional stability constant ($\bar{K}'$), was determined from a Scatchard analysis of data between UO_2^{2+} concentrations of 5.25×10^{-8} $\underline{M}$ (12.5 ppb) and 2.10×10^{-7} $\underline{M}$ (50 ppb) UO_2^{2+}. These concentrations fall within the range of surface waters in a non-uraniferous area (Szalay and Samsoni 1969). The Scatchard analysis over this range of metal:ligand ratios is sufficiently linear to allow the estimation of $\bar{K}'$ which was determined to be 2.43×10^{7} (or log $\bar{K}'$ of 7.38) from a single component Scatchard analysis (Figure 4 and Equation 22).

$$\frac{\bar{v}}{[M_f]} = 2.43 \times 10^7 \bar{v} + 2.65 \times 10^6 \qquad (22)$$

where: $\bar{v}$ = [ML]/BC

[ML] = molar concentration of complexed metal

BC = maximim binding capacity

$[M_f]$ = molar concentration of uncomplexed metal (UO_2^{2+}).

Table 3. Summary statistics for nonlinear least squares analysis to estimate the mean (μ) and standard deviation (σ) of uncomplexed UO_2^{2+} (M_f) and observed values of UO_2^{2+} complexed by humic acid (ML).

Source	DF	Sums of Squares (SS)	Mean Square (MS)
Regression	2	2.85×10^{-12}	1.42×10^{-12}
Residual	14	1.78×10^{-14}	1.27×10^{-15}
Uncorrected total	16	2.87×10^{-12}	
(Corrected total)	15	1.28×10^{-12}	

Parameter	Estimate	Asymptotic Standard Error	Asymptotic 95% Confidence Interval	
			Lower	Upper
K*	2.64×10^{5}	2.18×10^{4}	2.18×10^{5}	3.11×10^{5}
BC**	1.14×10^{-6}	5.0×10^{-8}	1.03×10^{-6}	1.25×10^{-6}

Asymptotic Correlation Matrix of the Parameters

	K	BC
K	1.000	-0.877
BC	-0.877	1.000

---

*K is a curve-fitting constant.

**BC is the maximum possible number of binding sites.

Figure 3. Scatchard plot of Aldrich humic acid binding of UO_2^{2+} at concentrations ranging from 4.2 x 10^{-8} $\underline{M}$ UO_2^{2+} (pH 4.5; 3.5 mg C/ℓ; 0.01 $\underline{M}$ Ac^-). $\underline{M}$ to 4.2 x 10^{-8} $\underline{M}$ to 2.1 x 10^{-5} $\underline{M}$ UO_2^{2+}.

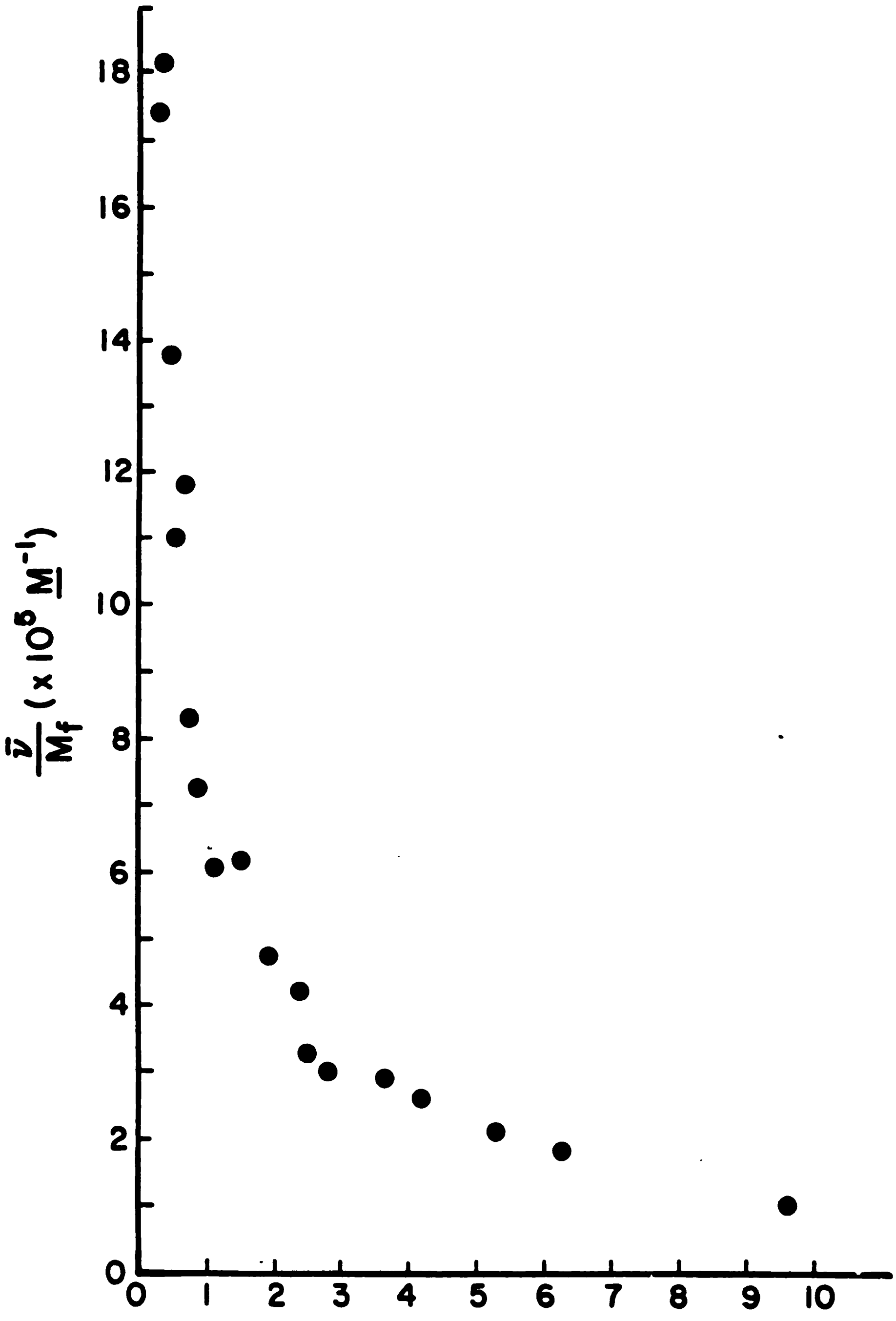

$\frac{\bar{\nu}}{M_f}$ (x 10^5 $\underline{M}^{-1}$)
0
2
4
6
8
10
12
14
16
18
0
1
2
3
4
5
6
7
8
9
10
$\bar{\nu}$ (x 10^{-1})

Figure 3.

Figure 4. The Scatchard estimate of the overall average conditional stability constant ($\bar{K}'$) in the region of interest based on the ratio of complexed uranium to maximum number of binding sites for Aldrich humic acid ($\bar{v}$) and uncomplexed UO_2^{2+} (M_f).

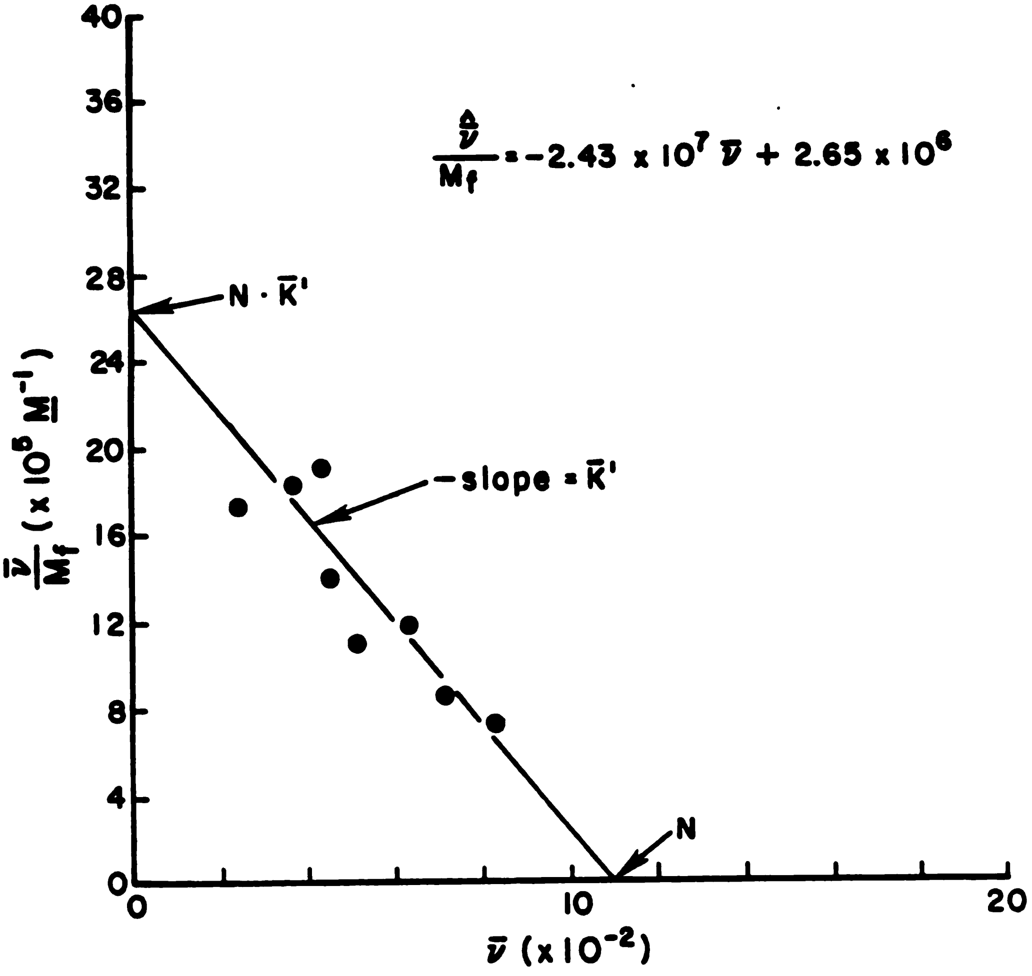

$\frac{\hat{\bar{\nu}}}{M_f} = -2.43 \times 10^7 \bar{\nu} + 2.65 \times 10^6$
N · $\bar{K}'$
−slope = $\bar{K}'$
N
$\frac{\bar{\nu}}{M_f}$ (x 10^5 M^{-1})
$\bar{\nu}$ (x 10^{-2})
0
4
8
12
16
20
24
28
32
36
40
0
10
20

Figure 4.

A Gaussian estimate of $\bar{K}'$ was determined to be 7.11×10^6 (or log $\bar{K}'$ of 6.85) for the UO_2^{2+} concentrations of 5.25×10^{-8} $\underline{M}$ to 2.10×10^{-7} $\underline{M}$ (Figure 5). The standard deviation ($\sigma_{\bar{K}'}$) of the estimated distribution of stability constants was calculated to be 1.38×10^6 (or log $\sigma_{\bar{K}'}$ of 6.14). The standard error of the estimate of $\bar{K}'$ was 4.6×10^5 (95% CI for $\bar{K}' = \pm 1.0 \times 10^6$; n=8). The logs of the observed conditional stability constants were plotted as a function of the log $[UO_2^{2+}]$:BC ratio. The log of observed $\bar{K}'$ as a function of log $[M_T]:[L_T]$ ratio shows that as the UO_2^{2+} ion is added, successively weaker sites are filled (Figure 5 and Equation 23).

$$\log \bar{K}' = -0.6913 \left(\log \frac{[M_T]}{[L_T]}\right) + 5.4 \qquad (23)$$

where: $\bar{K}'$ = overall conditional stability constant

$[M_T]$ = total concentration of UO_2^{2+}

$[L_T]$ = total concentration of HS binding sites.

Figure 5. Regression of the observed conditional stability constant (K') as predicted by a Gaussian distribution in the region of interest as a function of the metal (UO_2^{2+}) to ligand (Aldrich humic acid) ratio.

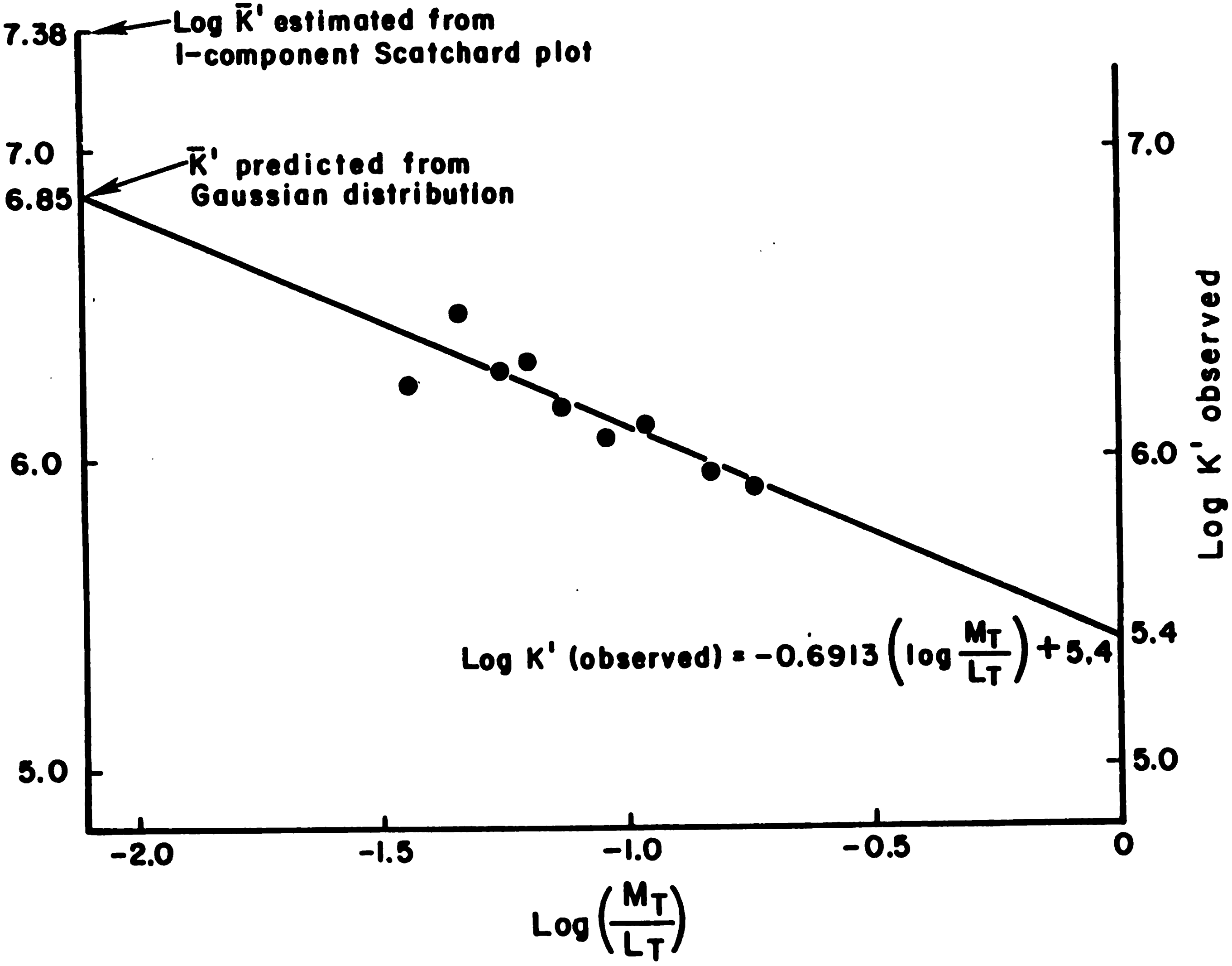

Log $\bar{K}'$ estimated from
1-component Scatchard plot
$\bar{K}'$ predicted from
Gaussian distribution
7.38
7.0
6.85
6.0
5.0
7.0
6.0
5.4
5.0
Log K' observed
Log K' (observed) = −0.6913 $\left(\log \frac{M_T}{L_T}\right)$ + 5.4
-2.0
-1.5
−1.0
−0.5
0
Log $\left(\frac{M_T}{L_T}\right)$

Figure 5.

DISCUSSION

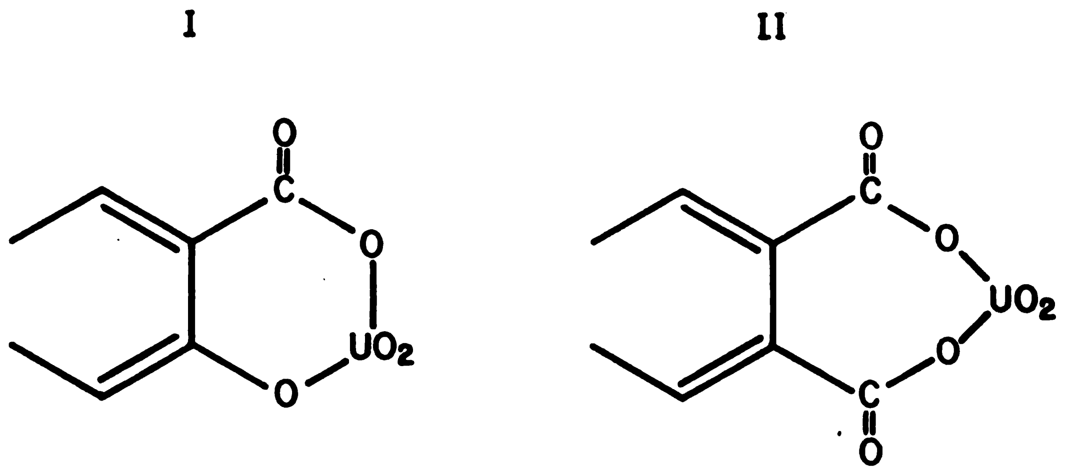
Typical inland surface waters of the coastal plain of the southeastern U.S.A. include swamps with dense vegetation and streams and rivers which drain highly leached, low relief terrain. These waters are regionally termed blackwaters and have pH values ranging from 3.8 to 6.8 and low ionic strengths. Their brown color is due to naturally occurring, refractory, organic compounds, known collectively as humic substances (HS). HS are a diverse group of polycarboxylic, polyphenolic compounds with both aromatic and aliphatic components which are believed to result from microbial degradation and chemical polymerization of former vegetative components such as lignin (Flaig 1964; Trojanowski et al. 1977; Haider et al. 1978), but are resistant to complete microbial degradation (Christman and Ghassemi 1966; Felbeck 1971).

Humic acids (HA) have been operationally defined as naturally occurring organics which are water soluble in basic solutions; fulvic acids (FA) are those refractory, colored organics that are water soluble in acidic or basic solutions. FA are relatively small organic molecules with nominal molecular weights (MW) ranging from 300 - 2000 (0.0009 - 0.0012µm). HA range from 5000 - 100,000 MW (0.0013 - 0.0052 µm) (Steelink 1977).

Several investigators (Schindler and Alberts 1974; Giesy and Briese 1978a) have reported that in low pH surface waters HS span a wide range of MW, but southeastern blackwaters generally are characterized by a high percentage of very small MW HS. Giesy and Briese (1977) report that 70% of the organic carbon (C) content of the Okefenokee Swamp water is < 0.0009 μm with an additional 27% in the next smallest size fraction (0.0009 μm $< F \leq 0.0015$ μm). These same authors report that greater than 60% of the dissolved organic carbon (DOC; < 0.45 μm) in two South Carolina streams is < 0.0015 μm (Giesy and Briese 1978a). Alberts and Evans (1979) show that greater than 80% of the DOC in four southeastern coastal plain rivers is < 0.0013 μm in diameter. Because of their prominence, FA fractions of HS may be a controlling variable in the mobility of trace metals in southeastern surface waters.

The importance of HS in the geochemical cycling of inorganic elements in surface waters of the southeastern U.S.A. has been established (Beck et al. 1974; Schindler and Alberts 1974; Casagrande and Erchull 1976; Reuter and Perdue 1977; Giesy and Briese 1977, 1978a). Despite the absence of descriptions of absolute structures for HS, there is general concensus that two functional groups are responsible for much of the complexing of metals in these freshwater systems. The carboxylate and phenolate groups occur in both aliphatic and aromatic configurations. While early research on HS supported a structure with an aromatic carbon backbone (Christman and Ghassemi 1966; Langford et al. 1983), more recent investigations suggest a less aromatic, more aliphatic structure (Stuermer and Payne 1976; Wilson and Goh 1977; Ruggiero et al. 1979, 1980). The phthalate and salicylate configurations of HS have been proposed as the most likely to chelate metals (Figure 6). Metal ions may be complexed by carboxylate and/or phenolate groups on single or two different humic molecules.

Figure 6. Types of metal complexation hypothesized for humic acid and fulvic acid (redrawn from Manning and Ramamoorthy 1973).



I

Salicylic Acid Group

II

Phthalic Acid Group

Figure 6.

Acid dissociation constants (K_a) for the two major types of exchangeable proton sites on humic molecules are similar to the K_a values of phthalic acid and salicylic acid (Table 4). More recent thermodynamic data tend to support salicylate type bonding, but not phthalate type bonding (Choppin and Kullberg 1978). Carboxylate groups attached to different aromatic rings may act independently of each other, not in an ortho-carboxylic (i.e., phthalate) configuration (Choppin and Kullberg 1978; Alberts and Giesy 1983).

Since HS are the principal ligands found in southeastern surface waters and the complexing of UO_2^{2+} ions by humics has been reported to be strong relative to competing ligands (Lamar 1968; Kribek and Podlaha 1980), the purpose of this study was to investigate the binding of UO_2^{2+} by HS under pH and ionic strength conditions that simulate those found in inland surface waters of the Southeast (Beck et al. 1974; Tilly 1975).

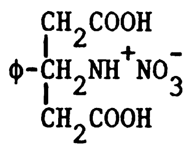
In natural waters metal complexation or adsorption can occur by interaction of UO_2^{2+} with different surfaces, such as ferric and manganic oxyhydroxides, inorganic anions, clays and organics (Moskvin et al. 1967; Langmuir 1978; Giblin 1980; Dongarra and Langmuir 1980; Borovec 1981; Giblin et al. 1981; Tipping 1981; Tsunashima et al. 1981). Since this study focused on UO_2^{2+}-HS complexation, other surfaces of adsorption and dissolved inorganic anions commonly found in surface waters were intentionally not introduced into the experimental solutions. For this reason, the results provide an estimate of the BC under laboratory controlled conditions and should not be interpreted as data that would result from field sampling.

Table 4. Acid dissociation (ionization) constants (pK_a) for two major types of proton sites on simple aromatic acids or aromatic acidic components of humic and fulvic acid molecules. Two or more constants indicate the first, second, and possibly the third dissociation constants, respectively.

Acid	pK_a	Study
benzoic	4.20	Perdue 1978
	4.01	Choppin & Kullberg 1978
phenol	9.95	Wilson & Kinney 1977
	9.78	Perdue 1978
phthalic	2.76; 4.92	Wilson & Kinney 1977
	2.7; 5.0	Choppin & Kullberg 1978
o-hydroxybenzoic	3.86; 13.1	Choppin & Kullberg 1978
(salicylic)	2.40	Gamble 1970
	2.97; 13.59	Wilson & Kinney 1977
	2.98; 13.59	Perdue 1978
m-hydroxybenzoic	4.1; 9.9	Choppin & Kullberg 1978
	4.52	Gamble 1970
p-hydroxybenzoic	4.5; 9.3	Choppin & Kullberg 1978
	4.52	Gamble 1970
	4.58; 9.24	Perdue 1978
humic/fulvic	3.1; 5.0; 5.0	Borggaard 1974
	4.0; 9.0	Choppin & Kullberg 1978
	4.23; 8.71	Wilson & Kinney 1977
	10.5 (pK_{ROH})	Perdue 1978

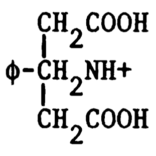
Kribek and Podlaha (1980) reported that the stability constant for the UO_2^{2+}-HS complex did not appear to depend on ionic strength, at least up to 0.5 M $NaClO_4$. In the study reported here, the ionic strength of the experimental solutions was an order of magnitude less than the ionic strength used by Kribek and Podlaha (1980; $I = 0.01$ M versus $I = 0.1$ M, respectively).

A weakly acidic catonic chelating resin (Chelex-100®) was selected for the study because of structural similarity between the resin matrix and solute (Aldrich HA solution). Chelex-100® is highly selective for divalent ions relative to monovalent cations. The exchange kinetics of Chelex-100® are governed by second order kinetics rather than diffusion. The aromatic nature of both the resin and the solute increase the probability that ion exchange, not adsorption, will occur. Chelex-100® has the ability to function in weakly acidic (pH $\geq$ 4), neutral or basic solutions. At low pH, Chelex-100® acts as an anion exchanger. The titration of Chelex-100® produces the following Zwitterionic forms as a function of pH:



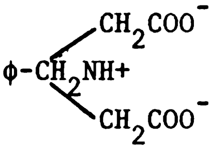
$\phi\text{-}\underset{\underset{\textstyle CH_2COOH}{|}}{\overset{\overset{\textstyle CH_2COOH}{|}}{CH_2}}NH^+NO_3^-$

pH 2.21

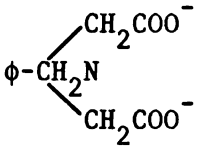


$\phi\text{-}\underset{\underset{\textstyle CH_2COOH}{|}}{\overset{\overset{\textstyle CH_2COOH}{|}}{CH_2}}NH+$

pH 3.99



pH 7.41



pH 12.30

At low pH some of the material in the HS solute may be complexed by the resin because of the anion affinity of the Chelex-100® resin and the net negatively charged characteristics of HS. The amount of loss of HS to the Chelex-100® was monitored by measuring the concentration of HS before and after passing through the column. At pH 4.5 of this study, Aldrich HA concentrations, as measured by fluorescence, ultraviolet absorption and CO_2 formation on combustion before and after passing through the column, were not significantly different (J. P. Giesy, pers. comm.). The loss of HA to the resin due to adsorption was small.

The Na^+ form of Chelex-100® will swell in water because of the hydration of functional groups. Therefore, when the resin was added to deionized water at pH 4.5, the pH of the water increased, indicating that the Chelex-100® resin had been protonated. Consequently, buffered solutions were used to maintain the experimental pH. An acetate buffer prepared from acetic acid (CH_3COOH) and sodium acetate (CH_3COONa) was selected because (1) the stability constant between UO_2^{2+} and the acetate anion (Ac^-) is small (Sillen and Martell 1964, 1971) and (2) the desired pH could be obtained by increasing the ionic strength of the experimental solution by less than two orders of magnitude over that reported for southeastern U.S.A. surface waters (Beck et al. 1974; Tilly 1975). The small stability constant between UO_2^{2+} and Ac^- permits the use of the acetate buffer without strong competition between Ac^- and aquatic HS for UO_2^{2+}. Other simple organic acids form stronger complexes with UO_2^{2+} and therefore might compete with HS for potential binding sites (Sillen and Martell 1964, 1971).

A 0.22 μm pore diameter Ac^- filter was placed over the glass-fritted section of each ion exchange column before the volume of buffered resin was added to prevent fragments of the resin from eluting into the filtrate. Van den Berg and Kramer (1979) noted that Chelex-100® fragments pass through a 0.45 µm filter and therefore could, in this study, increase the concentration of UO_2^{2+} in the filtrate. This would produce an overestimate of humic-bound UO_2^{2+} since it is presumed that all of the UO_2^{2+} in the eluate is bound to the HS instead of to HS and resin fragments. However, with the filters, very small pieces of Ac^- filter may pass through the fritted section of the column and into the sample; this would result in an increased carbon concentration in the sample. This error was greater than that resulting from fragments of UO_2^{2+} bound Chelex-100® resin passing through 0.45 µm filter (Table 5).

Calculations of binding capacities may also be affected by the kinetics of metal-ligand reactions (Giesy 1980). Because of greater affinity of the Chelex-100® for UO_2^{2+} ions, the solute flow rate must be sufficiently fast to prevent the resin from complexing humic bound UO_2^{2+} ions. If this does not occur, the results will not reflect the true binding potential of the HS for UO_2^{2+} and consequently the HS binding capacity will be underestimated. Conversely, if the flow rate through the ion exchange column is too fast, the Chelex-100® resin will not have sufficient opportunity to bind UO_2^{2+} not complexed by HS, and the binding capacity of the HS for UO_2^{2+} will be overestimated. An optimal flow rate of the metal-ligand solute must be established in order to approximate equilibrium conditions. In very similar columns, optimal flow rates of 4-6 ml/min have been reported in binding capacity studies with divalent cations and aquatic HS (Giesy 1980). However, because of the strong affinity of Chelex-100® resin for the

Table 5. Organic carbon analyses of replicate samples of dialyzed Aldrich HA (3.5 mg C/ℓ)

	Carbon concentrations in prepared solutions (ml C/ℓ)	
	Before elution through Chelex-100® column	After elution through Chelex-100® column
Without 0.22 µm Ac$^-$ filter	3.3	3.7
	3.4	3.6
With 0.22 µm Ac$^-$ filter	3.3	4.6
	3.4	5.3

UO_2^{2+} ion, flow rates between 17.5 and 18.8 ml/min were required in this study (Table 1).

The optimal flow rate was determined by eluting the buffered solute containing UO_2^{2+} and Aldrich HA through the Chelex-100® resin column at progressively faster rates. The point at which the UO_2^{2+} concentration reached a plateau as the flow rate continued to increase was defined as the optimal flow rate (Figure 2). At this flow rate uncomplexed UO_2^{2+} was removed from solution without allowing enough contact time for UO_2^{2+} complexed to HA to be removed. Partitioning complexed metal species from uncomplexed metal species by ion exchange can only be used when studying metal-ligand interactions with relatively high stability constants.

Several studies of the surface waters in the southeastern U.S.A. indicate HS have functional groups that are primarily carboxyl groups with fewer phenolic groups; Aldrich HA acidity, in contrast, is largely phenolic (Table 6; Beck et al. 1974; Reuter and Perdue 1977; Perdue 1979; Giesy and Alberts 1983). Although not necessarily representative of HS in all inland surface waters of the Southeast, Aldrich HA was used in this study instead of natural aquatic HS for several reasons. Aldrich HA has been used as a "reference" HA in other studies of BC and conditional stability constants (e.g., Pott et al., in press). Because it is commercially available and identified by lot number, Aldrich HA allows direct comparison of results to those of other metal-humic binding studies, whether the same or a different metal is studied. In addition, the same HA can be used for subsequent studies of UO_2^{2+}-HA complexing under different conditions, thereby eliminating one potential variable. The ash content of this

Table 6. Acidities of some oxy-functional groups in humic substances (meq/g; from Giesy and Alberts 1984).

	Total Acidity	Carboxylic Acidity	Phenolic Acidity
Soil HA[1]	7.9 ± 1.8[4]	3.7 ± 1.4	4.2 ± 1.5
Soil FA[1]	12.8 ± 1.2	8.9 ± 0.3	3.9 ± 1.6
Water HS[2]	10.4 ± 1.3	6.0 ± 1.3	4.4 ± 0.0
Upper Three Runs Creek[3] 12.0 nm $\geq$ HS $\geq$ 0.9 nm	4.2 ± 0.4	3.3 ± 0.4	1.0 ± 0.4
Aldrich humic acids	2.91 ± 0.25	0.42 ± 0.14	2.49 ± 0.29

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[1] Schnitzer and Khan, 1972

[2] Perdue, 1979

[3] HS = humic substance isolated by ultrafiltration

[4] ± 1 S.D. (N = 5, 3, 2, 2 and 17, respectively)

particular lot of Aldrich HA is 67%, which is comparable to the ash content of naturally occurring aquatic HS in a blackwater stream in South Carolina for which the ash content is 60-70% (Giesy and Briese 1977).

Despite the fact that numerous techniques are available to quantify uranium concentrations in aqueous solutions, only a few are sensitive enough to accurately quantify those U concentrations usually found in surface waters (Table 7). Most methods used a preconcentration or separation step which improves the minimum detection limit and eliminates interfering components in the solute (Campen and Bächmann 1979). Fluorometry is a sensitive analytical method for U and several variations of the fluorometric method have been devised (Table 7). Serious interferences in fluorometric analyses of U from natural waters is caused by the presence of HS (Robbins 1978; Campen and Bächmann 1979; Kaminski et al. 1981).

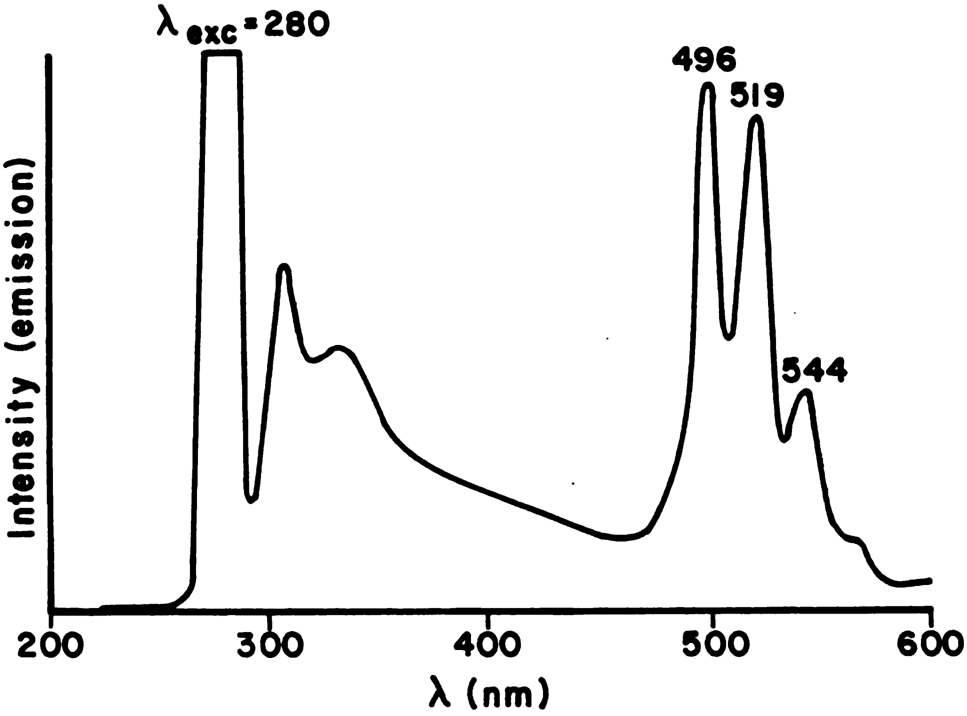
Recently a new fluorometric technique which overcomes DOC interference has been developed for the determination of trace U concentrations in surface waters. It has been tested extensively in the National Uranium Resource Evaluation (NURE) Program. The technique uses a Scintrex Model UA-3 laser fluorometer (Scintrex Ltd., Toronto, Canada) to measure laser-induced fluorescence of UO_2^{2+}. A nitrogen laser emits short intense pulses of ultraviolet radiation (UV) (λ = 337 nm) that excite the UO_2^{2+} ion. When excited by UV light, UO_2^{2+} compounds fluoresce in the green region of the spectrum with characteristic emission peaks at 496, 519, and 544 nm (Figure 7 from Campen and Bächmann 1979).

Table 7. Analytical techniques for low concentrations of uranium.

Analytical Technique	Minimum Detectable Limit (MDL)	Reference
Titrametric, micro-gravimetric	1.2 mg U/ml*	Kribek and Podlaha (1980)
Potentiometric	mg U/ml quantities	Bodnar (1980)
Gamma spectroscopy	1 mg/ml	Tieh et al. (1980)
	10 ng total U	Gladney et al. (1978)
	0.05 ng/ml	Gladney et al. (1976)
Colorometric (photometric)	0.6 mg/ml	Jablonski and Leyden (1978)
Liquid scintillation	0.1 mg/ml	Horrocks (1974)
Spectrophotometric	2 ng/ml*	Halbach et al. (1980)
	**	Nash et al. (1981)
Delayed neutron counting	0.5 ng/ml	Brits and Das (1978)
Fluorescence (fluorometric)	5 ng/ml	Li et al. (1980)
	2 ng/ml	Hathaway and James (1975)
	0.05 ng/ml	Campen and Bachmann (1979)
	0.05 ng/ml	Robbins (1978)
	0.04 pg/ml	Johnson and Wright (1981)
	0.01 pg/ml	Perry et al. (1981)
Phosphorescence (spectrofluorometric)	10 pg/ml	Kaminski et al. (1981)

*MDL not listed; concentrations listed are experimental concentrations
**No experimental concentration listed

Figure 7. Emission spectrum of an aqueous solution of uranium (from Campen and Bächmann 1979).



λ_{exc}=280
496
519
544
Intensity (emission)
200
300
400
500
600
λ (nm)

Figure 7.

Emission spectra of most UO_2^{2+} salts are quite similar, implying that the coordinating ligand has little effect on the strong O-U-O bonds (Robbins 1978). Intensity of the emission of various UO_2^{2+} species in solution varies considerably. Therefore, to insure maximum intensity of fluoresence, Fluran®, a proprietary reagent composed of a chelating agent and a fluorescing agent, is added to the UO_2^{2+} solution before laser excitation of the species occurs (Wallach et al. 1959; Campen and Bächmann 1979). The primary function of the Fluran® solution is the formation of a single fluorescent UO_2^{2+} species, but it also complexes other metallic ions in solutions to reduce their quenching effects on the intensity of the UO_2^{2+} luminescence (Robbins 1978). Fluran® strongly complexes the various UO_2^{2+} species in the solution into a single uranyl phosphate species and provides a high luminescent yield. Because the stability of the complex is pH dependent, Fluran® is sufficiently buffered to maintain a stable pH to insure the formation of a single species. Campen and Bächmann (1979) have reported signal intensity as a function of the volume of Fluran® added to the sample (Figure 8 from Campen and Bächmann 1979).

When excited by a nitrogen laser at 337 nm, HS exhibits an intense blue fluorescence with a maximum intensity near 400 nm (Figure 9 from Robbins 1978). Since the UO_2^{2+} ion fluoresces in the green region, any fluorescent interference from organic matter, which is emitted in the blue region, can be removed by using a green filter between the sample and the photomultiplier (Campen and Bächmann 1979).

Several other techniques were used to reduce interference. A standard additions technique was used to minimize matrix interference. In addition, a photo-oxidation procedure was used to remove

Figure 8. Relative signal intensity of Scintrex Model UA-3 fluorometer as a function of amount of Fluran® added to uranyl solution (from Campen and Bächmann 1979).

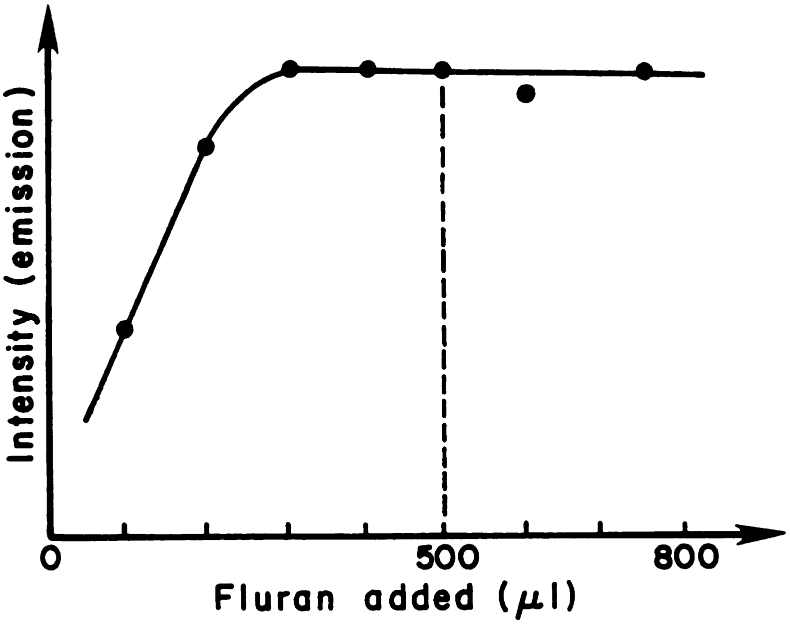


Figure 8.

Figure 9. Fluorescence spectrum of water samples containing humic substances (from Robbins 1978).

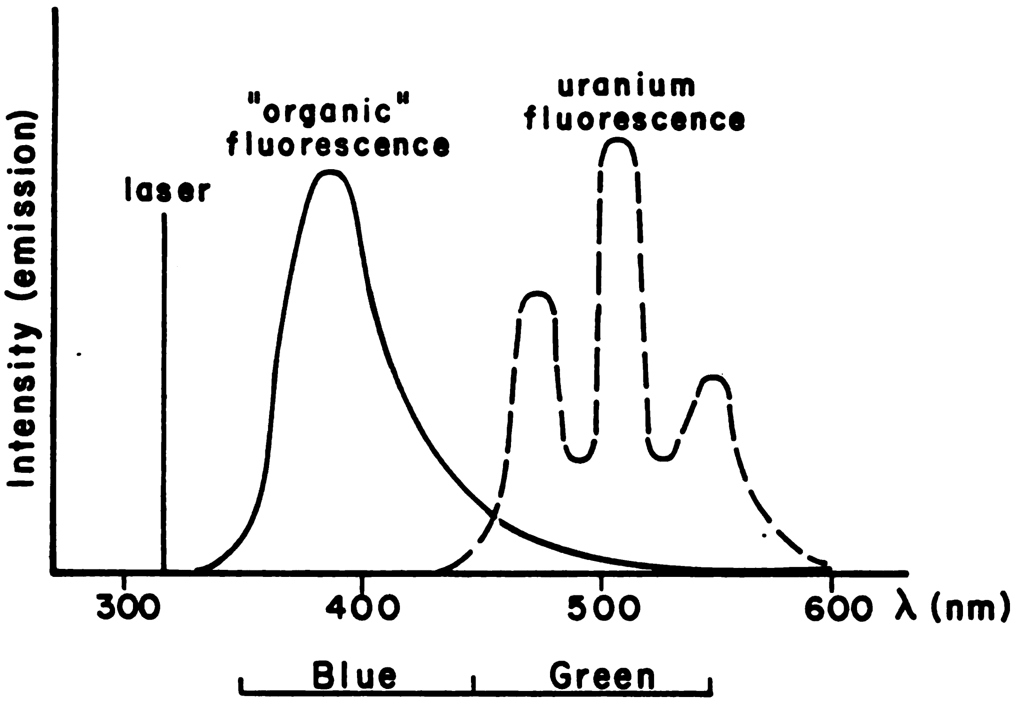

Intensity (emission)
laser
"organic" fluorescence
uranium fluorescence
300
400
500
600
λ (nm)
Blue
Green

Figure 9.

interference by HS. Carbon - carbon bonds in both humic molecules and acetate molecules are broken by high intensity UV radiation, in this study provided by a La Jolla Scientific Model PO-14 photo-oxidation unit. Other investigators have used photo-oxidation as a technique to destroy organic molecules and to release metals bound by organics (Blutstein and Shaw 1981; Sunda and Hanson 1979). This method is preferred over a permanganate oxidation procedure which supplants one interference (HS) with another (Mn^{2+}).

Laser fluorometry further reduces the potential interference due to fluorescence of HS by utilizing the differences in the lifetime of UO_2^{2+} fluorescence and that of humics in solution. The fluorescence of most naturally occurring organics ceases quickly. Lifetimes are, at most, several tens of nanoseconds. In contrast, fluorescence of dilute concentrations of UO_2^{2+} persist typically for several tens of microseconds (Robbins 1978). An electronic gating system, triggered by the laser, delays accepting signals from the photomultiplier until after the fluorescence from the humics has substantially ceased. The recorded signal is due almost entirely to the UO_2^{2+}.

Instrument response is generally very rapid after the addition of Fluran® to a solution that contains U; one exception is in the case of organic-rich samples. The extraction of U from organic species is slow (Robbins 1978). Analytical times for samples with greater concentrations of HS must be increased or samples with higher concentrations of HS must be pretreated with Fluran® several minutes before analysis. The fact that a significant amount of time is required for a combined chelating-fluorescing reagent to extract U from organic molecules provides further evidence that the stability constant between UO_2^{2+} and HS is large. A combination of laser fluorometry and photo-oxidation procedures provides a quick method to determine several binding characteristics of aquatic HS for UO_2^{2+}.

The BC calculated by fitting a hyperbolic function to the observed data is roughly 2.5 times greater than the BC estimated as a function of flow rate through the ion exchange column. The estimated BC is comparable to those reported for other divalent and trivalent cations. Alberts et al. (in press) 1983 note that the range of BC values for several metals is remarkably small for various waters of the eastern U.S.A. Giesy et al. (1978) concluded that BC of Cu^{2+} in surface waters of Maine was almost entirely controlled by organic components. Trivalent aluminum (Al^{3+}) strongly outcompetes the divalent ions Cd^{2+} and Pb^{2+} for binding sites on organic matter in surface waters (Alberts and Giesy 1983). Pott et al. (in press) observed that Aldrich HA solutions (2 mg C/ℓ) bound 8.9 and 9.6 x 10^{-7} $\underline{M}$ Al^{3+} at pHs of 4 and 5, respectively. In this study, the estimated BC of 1.4 x 10^{-6} $\underline{M}$ UO_2^{2+} was determined at an Aldrich HA concentration of 3.5 mg C/ℓ at pH 4.5. These data suggest that for Aldrich HA solutions at concentrations of 2 - 3.5 mg C/ℓ and pHs 4 - 5, the BC for Al^{3+} and UO_2^{2+} are similar. This would not be expected if the comparison is based on ionic charge alone.

Several factors may contribute to the fact that the BC of Al^{3+} and UO_2^{2+} by Aldrich HA are so similar. Effective radius of the U atom (1.38 x 10^{-10} m) is much larger than the hexavalent uranium ion (8.0 x 10^{-11} m). Because U is a very large atom, its nucleus holds electrons in the outer shells less tightly than those of the inner shells. Electrons in the outer shell can be removed from the U nucleus by more electronegative elements, such as oxygen (O^{2-}) in the case of uranyl ion (relative electronegativities: U^{6+}, 1.7; O^{2-}, 3.5; Brownlow 1979). This explains the ionic character of UO_2^{2+}. For comparison, the ionic radius of Al^{3+} has been reported as 5.1×10^{-11} m (Krauskopf 1967). Both Al^{3+} and UO_2^{2+} exhibit approximately the same percentage of ionic character (60 and 62%, respectively; Krauskopf 1967). Highly charged cations deform electron clouds of anions and consequently form mixed bonds (i.e., covalent and ionic). Because of their tendency to form covalent bonds as well as ionic bonds in water, ions such as U^{6+} and Al^{3+} distort electron clouds so that the relative strength of the metal-oxide and metal-hydroxide bond is no longer a simple matter of charge and radius (Krauskopf 1967). Electron cloud distortion by U^{6+} and Al^{3+} may also be true for other components in surface waters such as HS.

The conditional stability constant (K') is smaller for UO_2^{2+}-FA than for FE^{3+}-FA. That implies that UO_2^{2+} probably forms an inner sphere complex similar to the one between Fe^{3+}-FA (Gamble et al. 1976; Kerndorff and Schnitzer 1980). In an inner sphere complex, ligand functional groups may displace strongly coordinated water molecules and fill the vacated position in an inner sphere complex. By contrast, in an outer sphere complex, the ion is bound electrostatically to the ligand without displacement of coordinated water molecules (Kerndorff and Schnitzer 1980). Mn^{2+} does not chelate humic materials, but is bound in fully hydrated form with FA in an outer sphere complex (Gamble et al. 1976, 1977). At pH 4.7, Kerndorff and Schnitzer (1980) found the following order of sorption of metals onto FA:

Hg = Fe = Pb = Cu = Al = Cr > Cd > Ni = Zn > Co > Mn.

Disnar (1981) showed that UO_2^{2+} outcompeted Cu^{2+} for binding sites on a humic molecule, regardless of the order of introduction. Reactions of HA with UO_2^{2+} in the presence of other divalent cations have shown that HA has a higher cation exchange capability for UO_2^{2+} than for other metal cations (Halbach et al. 1980). It has been demonstrated that the selectivity of HA for several cations diminished in the order $UO_2^{2+} > Mn^{2+} > Ca^{2+} > H^+$ (Halbach et al. 1980).

Only a few values have been reported for the conditional stability constant of the interaction of UO_2^{2+} with HS. Kribek and Podlaha (1980) reported conditional stability constants (K') for UO_2^{2+} -HS complexes estimated by a microgravimetric procedure but did not postulate on the mode of UO_2^{2+} -HS binding. While their results are similar to those of this study (Table 8), the freezing of extracted HS by Kribek and Podlaha (1980) may have altered physical and chemical properties of the organics since HS may aggregate into particulates after freezing (Lush and Hynes 1973; Giesy and Briese 1978b).

Li et al. (1980) reported on the effect of pH and UO_2^{2+}: ligand ratio on the binding of UO_2^{2+} by HS. They reported two types of binding sites determined by molecular fluorescence spectrometry with a difference of about two orders of magnitude between the stability constants of the weaker and stronger binding sites with the stronger binding sites representing only about 10% of the total number of available binding sites. While their study contains useful information, the experimental conditions do not typify the natural

Table 8. Average conditional stability constants ($\bar{K}'$) for aqueous complexes of UO_2^{2+} ions and humic substances.

Ligand	pH	Ionic Strength (I)	log $\bar{K}'$*	Reference
Humic Acid (HA)	6.0	0.1 $\underline{M}$ KNO_3	6.37	Li et al. (1980)
Fulvic Acid (HA)	6.0	0.1 $\underline{M}$ KNO_3	7.43	Li et al. (1980)
Tannic Acid (TA)	6.0	0.1 $\underline{M}$ KNO_3	6.36	Li et al. (1980)
HA	2.7 - 6.5	0.1 $\underline{M}$ $NaClO_4$	7.8 ± 0.4	Kribek and Podlaha (1980)
HA	2.9 - 6.8	0.1 $\underline{M}$ $NaClO_4$	7.7 ± 0.3	Kribek and Podlaha (1980)
HA	3.1 - 7.0	0.5 $\underline{M}$ $NaClO_4$	7.7 ± 0.2	Kribek and Podlaha (1980)
HA	4.5	0.01 $\underline{M}$ CH_3COONa, CH_3COOH	7.38 (Scatchard analysis)	this study
HA	4.5	0.01 $\underline{M}$ CH_3COONa, CH_3COOH	6.85 (Gaussian analysis)	this study

$$* \; \bar{K}' = \frac{[UO_2L]}{[UO_2^{2+}][L^-]^2}$$

situation in which uranium concentration remains relatively constant while the aquatic HS concentrations can vary greatly, depending on season and amount of rainfall. When the entire range of total UO_2^{2+} concentrations investigated by Li et al. (1980) is presented as a Scatchard plot, a two-component plot is obtained similar to the one presented in Figure 3. This nonlinear response suggests multiple binding sites.

Halbach et al. (1980) discussed the uptake of U as a function of total acidity and noted that at pH 4.5, all acid groups were dissociated into carboxylates or activated phenolates or were at least easily dissociable. Because HS are weak acid cation exchangers, the uptake of UO_2^{2+} by HA and FA is an ion exchange process which is influenced by pH. Halbach et al. (1980) believe that both carboxylate and phenolic acid groups are able to bind equivalent quantities of UO_2^{2+} ions. This does not agree with the findings of Li et al. (1980).

A Scatchard analysis of the entire range of UO_2^{2+} concentrations used in the current study (4.2×10^{-8} $\underline{M}$ to 2.1×10^{-5} $\underline{M}$ UO_2^{2+}) also produced a nonlinear plot but with significantly larger x- and y-intercepts than Li et al. (1980). The number of total binding sites ($n_1 + n_2$) varied significantly between this study and Li et al. (1980), indicating that the concentration of humic-complexed UO_2^{2+} also differed. This was probably because some of the smaller MW organics complexed UO_2^{2+}, but still passed through the dialysis membrane. Li et al. (1980) maintain that 100% of U^{6+} (i.e. UO_2^{2+}) is uncomplexed by FA (MW from 300 - 2000) and even smaller MW tannic acids at pH 3 and 4, respectively. These investigators maintain that the only dialyzable species was the uncomplexed uranyl ion, UO_2^{2+}, since the nominal MW exclusion limit for the dialysis membrane was 3500. What they report as uncomplexed UO_2^{2+} (dialyzable U^{6+} or M_f) may in fact contain some organically bound UO_2^{2+} (ML) if the MW of the organic fraction is smaller than about 3000 nominal MW units.

In surface waters of the southeastern U.S.A. most of the DOC is composed of the smaller MW fulvic acids (Beck et al. 1974). Nearly half (48%) of the aquatic HS in a blackwater creek in South Carolina has a MW of less than 500 (J. J. Alberts, pers. comm.). Halbach et al. (1980) concluded that the UO_2^{2+} ion migrates generally as dissolved uranyl fulvates in flowing surface waters and that the migration is favored by a humid climate with high annual rainfall. The coastal plain of the southeastern U.S.A. is characterized by similar climatic conditions.

The possibility that the UO_2^{2+} inside the dialysis membrane was associated with very small MW organics was not considered by Li et al. (1980). In actuality, the stability constant that they reported may be significantly lower than the true stability constant because the binding potential of the very small HS was not considered. The assumption that no UO_2^{2+} was complexed by the small MW organics also led to a diminished estimate of the complexed UO_2^{2+}:uncomplexed UO_2^{2+} ratio (y-axis). This ultimately produced an underestimate of the product of the conditional stability constant (K') and the number of binding sites (n), since n had been underestimated previously. It should be noted that the present study was performed at a lower pH (4.5) than that of Li et al. (1980; pH 6.0). The different ionic strengths probably do not account for significantly different results, however.

Perdue and Lytle (1983) have questioned whether experimental results from Scatchard analyses of high metal-ligand concentrations can be extrapolated beyond the limits of the study to very low metal-ligand concentrations which generally typify natural waters. They reported that values in the Gaussian model are still rising sharply at ligand-metal concentrations ($[L_T]:[M_T]$) greater than 100 ($\log [L_T]:[M_T] \geq 2$). Because laser fluorometry is a very sensitive analytical technique, unrealistically high UO_2^{2+} concentrations were not necessary to measure the complexed UO_2^{2+} ion. Thus the uncertainty expressed by Perdue and Lytle (1983) was not a consideration. In fact, this study extended the theoretical work of Perdue and Lytle (1983) to an actual metal-ligand system.

A continuous distribution model such as the Gaussian model does not account for mass balance so care must be taken during experimental design to obey laws of mass balance. Perdue and Lytle (1983) state that in natural waters with 10 mg C/ℓ of aquatic HS and an $M_T = 10^{-8}$ $\underline{M}$, $\log L_T/M_T = 3$, the Scatchard analysis underestimates K' by several orders of magnitude. The gradual change of K' characterized by the Gaussian distribution model cannot be modelled by the Scatchard equation at high $[L_T]:[M_T]$ ratios typical of natural surface waters (Perdue and Lytle 1983). It should be noted that 10 mg C/ℓ of aquatic HS is not considered a great concentration of organic material for surface waters of the southeastern U.S.A. (Beck et al. 1974; Giesy and Paine 1977; Reuter and Perdue 1977; Giesy and Briese 1978a; Alberts and Evans 1979). Neither the Scatchard model nor the Gaussian model has been reported to be very effective at describing the experimental data at both low $[L_T]:[M_T]$ values common to most laboratory studies and the high $[L_T]:[M_T]$ ratios that typify natural surface waters (Perdue and Lytle 1983). However, the current study shows that when the conditional stability constant is determined in the $[L_T]:[M_T]$ range representative of environmental concentrations, the Scatchard estimate and the Gaussian estimate of K' are similar (2.4×10^7 and 7.1×10^6, respectively). These findings are the reverse of those reported by Perdue and Lytle (1983) who stated that the Scatchard method underestimated log K' by several orders of magnitude.

CONCLUSION

Natural humic substances (HS) can complex uranium from concentrations similar to those of surface waters of non-uraniferous areas. Under simulated conditions of surface waters for the southeastern U.S.A. (pH 4.5 and I = 0.01 $\underline{M}$), a laser fluorometric procedure was used to determine binding capacity (BC) and average conditional stability constant ($\bar{K}'$) of Aldrich humic acid (HA) for the uranyl ion (UO_2^{2+}).

At pH 4.5 and I = 0.01, Aldrich HA (3.5 mg DOC/ℓ) has a BC of 1.4 x 10^{-6} $\underline{M}$ UO_2^{2+} comparable to the BC of Aldrich HA determined for Al^{3+} under similar laboratory controlled conditions (Pott et al., in press). A highly charged cation such as UO_2^{2+} may be able to deform electron clouds of Aldrich HA and can consequently form mixed bonds (i.e., covalent and ionic bonds). Aldrich HA may then chelate the UO_2^{2+} ion rather than bind it electrostatically and subsequently displace strongly coordinated water molecules.

Average conditional stability constants ($\bar{K}'$) for UO_2^{2+} to Aldrich HA were estimated by a graphical method (Scatchard plot analysis) and from a frequency distribution (Gaussian distribution). Both the Scatchard and Gaussian methods resulted in similar estimates for log $\bar{K}'$ (7.38 for single component Scatchard plot and 6.85 for the continuous Gaussian distribution).

From this study it can be concluded that the Gaussian estimate is not necessarily superior to the Scatchard estimate of the average conditional stability constant, $\bar{K}'$, provided certain conditions are met. If metal concentrations $[M_T]$ used in the simulation studies are truly representative of environmental concentrations of the metal of interest, it does not appear that a great difference exists between the K' estimated by the two methods. However, if the Scatchard plot is used to calculate K', one should not attach chemical significance to the four empirical curve-fitting parameters. If higher experimental metal concentrations are necessary than those typical of natural surface waters, preliminary results suggest that a Gaussian distribution model is more accurately representative than the two-component Scatchard equation in its ability to predict accurate log K' values (Perdue and Lytle 1983).

A plot of the observed log K' as a function of the $[L_T]:[M_T]$ ratio indicates a successive filling of sites (Li et al. 1980). Thermodynamically more stable sites are filled first with less stable sites filled later. An alternative but less probable interpretation of this observation is that as sites are filled the nature of the remaining sites changes such that they bind metal less tightly. This observation has implications for both estimating conditional stability constants (K') and applying them in geochemical models. Under conditions of small $[M_T]:[L_T]$ ratios common for dilute solutions of UO_2^{2+}, only the strongest sites would participate in binding. Estimates of K' made at greater $[M_T]:[L_T]$ ratios would certainly be overestimated.

LIST OF REFERENCES

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